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A TEXT-BOOK

OF THE

PHYSIOLOGICAL CHEMISTRY

OF THE

ANIMAL BODY

INCLUDING AN ACCOUNT OF THE CHEMICAL CHANGES OCCURRING IN DISEASE

BY

ARTHUR GAMGEE, M.D., F.R.S.,

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WITH TWO CHROMO-LITHOGRAPHIC CHARTS BY SPILLON AND WILKINSON.

VOL. II.

THE PHYSIOLOGICAL CHEMISTRY OF DIGESTION.

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PREFACE.

THE first volume of this work which appeared in the year 1880 formed a complete and independent treatise on the physiological chemistry of the elementary tissues of the animal body, including under this designation the blood, the lymph, and the chyle.

The present volume, like its predecessor, constitutes an independent and complete treatise; it deals with the physiological chemistry of the digestive processes, which have been treated on the same lines as were followed in Vol. I. My aims and endeavours are clearly set forth in the following sentences which appeared in the preface to that volume:

'It has been a constant object with me to give the reader a very full and, so far as possible, independent account of the state of knowledge on the subjects discussed, and I trust I may with complete truthfulness say that this work is based upon a study of original memoirs, rather than upon a study of text-books. In the interest of the student, nearly all papers are quoted by their full titles and few have been quoted which have not been thoroughly read and studied. Whenever quotations have been made at second hand the fact has been stated.'

'Another feature which I have desired to render prominent in this work is the description of the methods which have been followed in important and, to borrow a convenient Germanism, 'epoch-making' researches. It seemed the more important to do this as I desired to write in the interest of the truly scientific student, anxious not merely to learn what has been already acquired to science, but wishful himself to extend her boundaries.'

'I have, as far as possible, tried all the experimental processes mentioned in this book, and throughout it I have incorporated the results of my own independent researches which, in many cases, have not yet been published elsewhere.'

The publication of several excellent Manuals, such as those of Hammarsten and of Haliburton, which deal, in a comprehensive but necessarily succinct manner, with the whole field of Physiological Chemistry, have adequately met the wants of a large class of students whilst, I venture to think, they have left the field open for a work which shall be based on an original study of the whole literature of the subjects treated of, and which shall be an accurate guide to the advanced student and the original worker, both in the study and the laboratory.

As in the first volume, though in a more detailed manner, I have written not merely as a scientific chemist, but from the stand-point of the physiologist, and I have treated with especial care all subjects which are of interest to the pathologist, the pharmacologist, and the scientific physician.

In illustration, I may cite the chapters in which I discuss the pathology of jaundice, the pharmacology of icterogenic poisonous agents and of cholagogues, the structure and formation of gall-stones, the investigation of the gastric contents, &c.

If circumstances permit me, it is my wish after the publication of a second edition of Vol. I., to complete, by an equally thorough study of other great animal functions, my survey of the Physiological Chemistry of the Animal Body. Whether that wish be accomplished or not, I trust that the present volume may, like its predecessor, further the advancement of, and prove not altogether unworthy of the present position of, physiology in England.

I have to acknowledge my deep indebtedness to Professor Hugo Kronecker who has placed at the disposal of his old friend the whole resources of the Physiological Institute of the University of Berne, besides helping me by his profound acquaintance with the literature of physiology. I am also indebted to Professor Drechsel, who is as distinguished for his discoveries throughout the whole range of Physiological Chemistry as for his encyclopædic knowledge of its literature and who has aided me by most valuable suggestions and by the loan of papers and books. I would acknowledge my obligations to Mr F. J. H. Jenkinson, M.A., Fellow of Trinity College and Librarian of the University of Cambridge, for enabling me, during a residence in Cambridge, to make full use of the splendid library under his direction; to Professor Blösch, Librarian of the fine Stadt Bibliothek of Berne, and to the Authorities of the University Library of Berne for allowing me the freest use of the books under their charge.

Finally, I would appeal to my fellow-workers to aid me by communicating to me any errors in this book which have neither been noticed amongst the *corrigenda* nor corrected in the Appendix, and especially to forward to me copies of original papers bearing on the subject-matter of the present volume and especially of Vol. I., which is now occupying my attention.

ARTHUR GAMGEE.

PHYSIOLOGICAL INSTITUTE,
UNIVERSITY OF BERNE,
October, 1893.

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CORRIGENDA.

Page 129, six lines from bottom, for 'hetero-' read 'deutero-'.

,, 129, five lines from bottom, for 'hetero-' read 'deutero-'.

,, 131, six lines from bottom, for 'hemi-deutero-albumose' read 'anti-deutero-albumose.'

,, 181, first line from bottom, for '1859' read '1852.'

,, 216, seven lines from top, for 'VI.' read 'IX.'

,, 247, twenty-nine lines from top, for 'Para-oxyphenyl-α-propionic acid' read 'Para-oxyphenyl-propionic acid.'

,, 258, eight and twelve lines from top, for 'Lohr' read 'Loew.'

,, 258, thirteen lines from top, for 'Lorsch' read 'Lossen.'

,, 326, three lines from bottom, for 'Monatsch.' read 'Monatshefte.'

,, 364, in several places, for 'Städelmann' read 'Stadelmann.'

,, 471 reference omitted to paper by Michael Foster 'On the existence of Glycogen in the tissues of certain Entozoa.' *Proceedings of the Royal Society*, Vol. 14 (1865), p. 543.

BOOK II.

THE PHYSIOLOGICAL CHEMISTRY OF DIGESTION.

CHAPTER I.

INTRODUCTORY REMARKS ON THE ALIMENTARY JUICES GENERALLY.

SALIVA AND ITS ACTION UPON FOOD.

INTRODUCTORY OBSERVATIONS.

DIGESTION is the process whereby the constituents of the food are rendered soluble and converted into bodies which are capable of absorption. These constituents are in part mineral, and of these the chief undergo no important chemical change prior to absorption. The larger part consists, however, of complex carbon compounds, which are for the most part insoluble in water when ingested, and which, after suitable mechanical processes of division and trituration, are subjected to the action of certain digestive juices which dissolve them and render them diffusible.

The digestive juices the products of secreting glands.

The juices above referred to are produced in, or by the agency of, the epithelium cells lining the interior of the glands which are either situated in the walls of the alimentary canal or which empty their secretion into it. Although these cells derive the materials necessary for their metabolic activity from the blood, the substances which they elaborate, and which are characteristic of the secretion which they help to form, are not found in the blood, but are the products of the activity of the protoplasm of the cells themselves.

Enzymes or ferments of the alimentary canal.

The characteristic constituents of the several juices which are specially concerned in the chemical changes of the alimentary canal are certain so-called 'unorganised' ferments, which we shall, following the suggestion of Kühne, denominate *Enzymes*. These are capable, like other ferments, of initiating, under suitable circumstances, specific changes in certain bodies with which they are brought into contact, changes which may be incommensurably great when contrasted with the magnitude of the mass of the ferment engaged. These specific actions of ferments lead to the breaking down of complex into simpler molecules, the decomposition being necessarily associated with the conversion of some potential into kinetic energy, which usually appears as heat.

'*Unorganised*,' or as they have also been called, '*unformed*' ferments differ, however, from the '*organised*' or '*formed*' ferments in that, whilst they are the products of the activity of living protoplasm, they cease, after being formed, to have any necessary connection with organised forms, and have no power of reproduction or increase.

Certain enzymes exert their action unimpaired in the presence of certain bodies which act as poisons to and kill the great majority of organised ferments; thus salicylic acid and thymol, in not too great quantity, do not hinder peptic and tryptic digestion, but prevent the putrefactive changes which are very apt to occur in the latter case, and which depend upon the development of organised ferments. Certain enzymes, however, as the diastase of malt, or as the diastatic enzymes of saliva and pancreatic juice, are destroyed by salicylic acid.

Changes in secreting cells corresponding to variations in the functional activity of organs.

As will be shewn in detail in the sequel, the secreting cells of glands which produce enzymes exhibit marked differences or variations which correspond to different states of activity. In the case of the secreting cells of the pancreas, as was discovered by Heidenhain, the cells appear to produce and store up for a time a body, a so-called '*zymogen*,' from which an enzyme called '*trypsin*' is set free; similarly, as the researches of Ebstein and Grützner, Langley and others have shewn, the secreting cells of the gastric glands produce in the first instance an antecedent of pepsin which we may term '*pepsinogen*.' There is further reason to believe that the rennet-ferment has a corresponding zymogen. The progress of research will probably reveal the existence of *zymogens* in relation to other animal enzymes.

Enzymes or their 'zymogens' usually present in the secreting structures which form them and may be extracted therefrom.

Usually the glandular organs which produce the digestive juices contain stored up within them during the periods in which they are actively secreting their characteristic enzymes or their zymogens; these may be extracted by digesting the comminuted organ in water, weak spirit, chloroform-water or still better in glycerin, which dissolves them nearly all, and furnishes solutions which preserve their activity long unimpaired[1].

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[1] In reference to the solubility of enzymes and zymogens in glycerin, Mr Langley has furnished me with the following note:—'I do not think it proved that ferments or zymogens are soluble in *pure strong* glycerin. If they are soluble it is extremely slowly. If the œsophagus of a pig be dried and put in pure glycerin, in a well-stoppered bottle, it does not give one-sixtieth of its ferment (counting zymogen as

Enzymes are all insoluble in strong alcohol, so that the tissues from which they are to be extracted, having by mechanical means been reduced to as fine a state of division as possible, may be first dehydrated by placing them in absolute alcohol, and afterwards extracted with glycerin or other suitable solvent. The treatment with alcohol has for its object the rendering insoluble of proteids which would otherwise dissolve in the liquid employed for the extraction of the ferment and thus furnish a less pure solution.

Solutions of enzymes are, for the most part, rendered instantaneously inactive by boiling; exposure to a temperature of 70° C. also destroys their activity, though less rapidly, and prolonged heating at lower temperatures exerts the same effect, though the lower limit, which doubtless varies in the case of the different enzymes, has not yet been ascertained.

Nature of the action exerted by enzymes.

It has already been stated that under the influence of enzymes, the complex organic bodies which are susceptible to their action are decomposed, complex breaking up into simpler molecules. These ferments appear to possess the power of rapidly inducing, at the temperature of the animal body, chemical changes in bodies subjected to them which are similar in character to those which are brought about with great slowness by prolonged heating with dilute mineral acids, or by the prolonged action of boiling water or of superheated steam. These operations are of the nature of 'hydrolytic' decompositions, that is to say, such as are connected with the union of the elements of water with the body undergoing decomposition (see Vol. I. p. 19).

A complete treatment of the theory of ferment action, or rather an account of the views which have been held at various times in regard to the action of ferments, though of great interest to the student of scientific history, would require too lengthy a discussion. The subject is one, however, which cannot be passed over without some remarks.

The modern scientific history of ferments and their actions commences with the researches of Payen and Persoz[1] on Diastase, and those of Cagniard-Latour[2], and afterwards of Theodor Schwann[3], on Alcoholic Fermentation.

Three principal hypotheses have been propounded to account for ferment action:—of these the two first are still appealed to, to explain

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ferment) after a week. When a tissue has been ground it is impossible to separate the particles from the glycerin, and the particles of the pancreas pass readily through the finest filter paper. In most cases the glycerin extract has been simply strained through linen; sometimes it has been filtered, but then it is doubtful whether sufficient care has been taken to prevent the dilution of the glycerin; in dilute glycerin it is probably the water (or dilute salt solution) which is the solvent.'

[1] Payen et Persoz, 'Mémoire sur la Diastase,' *Annales de Chimie et de Physique*, Vol. 53 (1833), p. 73.

[2] Cagniard-Latour, 'Mémoire sur la Fermentation Vineuse, présenté à l'Académie le 13 Juin, 1837,' *Annales de Chimie et de Physique*, Tome 68 (1838), pp. 206—221.

[3] Schwann, 'Vorläufige Mittheilung betreffend Versuche über die Weingährung und Fäulniss,' Poggendorff's *Annalen*, Vol. 41 (1837), pp. 184—193. Refer also to his 'Microscopic Researches, &c.' *Sydenham Society*, 1847, p. 190.

the actions of unformed, whilst the third has exclusively reference to formed ferments.

1. The contact or 'catalytic' theory of Berzelius.
2. The modification of the catalytic theory formulated by Liebig.
3. The physiological theory, which now holds undivided sway and which owes its commanding position to the splendid researches of Pasteur. This theory considers every ferment process to be the resultant of the activities of a definite organism.

The theory of 'Catalysis' of Berzelius.

There are certain chemical reactions which occur between two bodies, in which the presence of a third exerts a remarkable influence, without the third body appearing on superficial examination to be modified by the process which it has helped to bring about. When, for instance, mixtures of hydrogen and oxygen find themselves in the presence of finely divided platinum, the two gases combine, under certain circumstances, with explosive violence. Again, when platinum black is brought into contact with the vapour of alcohol, the latter is oxidised and acetic acid is formed. To cite a third case, when peroxide of hydrogen, H_2O_2, is treated with platinum black, that very unstable compound breaks up into water and oxygen, it being obvious that in this case two molecules, at least, of the peroxide must be concerned, its results being the formation of two molecules of water and a molecule of oxygen. As was discovered long ago, not only is this remarkable reaction brought about by platinum and certain other inorganic bodies, but also by certain organic substances. A shred of fibrin, for instance, or a drop of blood, or of a solution of hæmoglobin will suffice to decompose a large quantity of a solution of hydrogen peroxide, which is thrown into effervescence and rises in temperature.

Berzelius was the first to bring together, and draw attention to, these remarkable phenomena, which he distinguished as 'catalytic' and explained as due to the action of a hitherto unrecognised force, to which he ascribed the term 'catalytic force[1].'

'It is then proved,' he remarked, 'that several simple and compound, soluble and insoluble, bodies, are capable of exerting upon certain other bodies an action which is very different from that of chemical affinity. By this agency they are the means of producing in those bodies decomposition of their elements, and subsequent rearrangements of the same without they themselves taking a part in them.

'This new force, which has hitherto been unrecognised, is common to organic and inorganic nature. I shall, therefore, call this force *catalytic force*. I shall, similarly, call *catalysis* the decomposition of bodies through the agency of this force.'

Amongst the most obvious examples of catalytic phenomena, Berzelius classed the actions of ferments, both formed and unformed. In criticising the theory of Berzelius we must appreciate that its essence consisted in the hypothesis that the catalysing agent remained absolutely passive, whilst no attempt was made to shew in what manner this remarkable contact action of the catalysing body operated. In the case of the most striking catalytic actions to which Berzelius drew attention, the progress of research has utterly disproved the passive part of the catalysing agent. The occlusion of gases by metals, for instance, is a process in which unstable molecular compounds are formed, and it plays, no doubt, a most important part in the cases where finely divided metals bring about the combination of gases. In these cases easily dissociated compounds are doubtless formed, and both the heat generated at the time of combination and the ease with which the newly-formed compound splits up with variations in temperature furnish the conditions which are necessary for the so-called catalytic processes.

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[1] Berzelius, 'Quelques idées sur une nouvelle force agissant dans les combinaisons des corps organiques.' (*Annales de Chimie et de Physique*, Tome 61 (1836), pp. 146—151).

There are obviously a variety of types of so-called catalytic processes. Essentially they are all processes in which are concerned, as the principal factors, bodies of which the constituent atoms and molecules are in a state of virtually unstable equilibrium. An *apparently* insignificant variation in the conditions under which they exist is sufficient to lead to a rearrangement of the molecules of which they are composed and to chemical transformations of the most striking characters, often associated with transformations of energy which are even more startling. To conclude, however, that the catalytic agent, which has furnished the energy which has as it were exploded the mine, remains actually passive, is to embrace a hypothesis which is opposed to all analogy.

The conception of a catalytic force absolutely indefinite in nature, and displayed by a body whose function is active but whose transformations are none, is, as suggested by Hüfner[1], nearly akin to the adoption of the conception of a vital force to explain obscure phenomena beyond the reach of actual knowledge. The doctrine of catalysis embodied, however, conceptions which are opposed to great and immutable principles. The true and, as it appears to the author, the really philosophical conception of the processes of catalysis was admirably set forth by the great J. R. Mayer.

'We call a force catalytic,' says the philosopher of Heilbron, 'when it holds no commensurable proportion to the assumed results of its action. An avalanche is hurled into the valley......a puff of wind or the fluttering of a bird's wings is the catalytic force which has given the signal for, and which is the cause of, the widespread disaster[2].'

The theory of catalysis of Berzelius possessed the sole merit of calling attention to a previously unstudied group of phenomena, which however it attempted to explain in a manner which did not tend to throw any light upon them.

Liebig's modification of the theory of 'Catalysis.'

Liebig[3] modified the Berzelian theory, especially in reference to the ferments, by supposing that a ferment is invariably a body in a state analogous to, if not identical with, decomposition, and that in virtue of the changes which it is itself undergoing it is able to bring about changes in the bodies subjected to its action. According to him, then, the body undergoing fermentation is, in a sense, inductively acted upon by the ferment, but the influence of the latter is a fortuitous one. Liebig believed the ferments to be essentially albuminous bodies, which acquire their ferment activities in virtue of their proneness to decomposition, which is so great a characteristic of these bodies in the presence of moisture and a suitable temperature. By this hypothesis, Liebig sought to explain the action of the formed, as well as of the unformed ferments, believing that the processes of life which are characteristic of the former led to the production of the very unstable substances, whose further putrefactive decomposition he held to constitute the first stage of any process of fermentation.

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[1] Hüfner, 'Zur Lehre von den katalytischen Wirkungen. Erste Abtheilung, 1. Ueber die geschichtliche Entwickelung des Begriffs.' *Journal f. prakt. Chemie*, Vol. 10, 1874, p. 148.

[2] J. R. Mayer, *Mechanik der Wärme*, 1867, p. 91, quoted by Hüfner. The author has been unable to verify this reference.

[3] Liebig, J. v. 'Rechtfertigung der Contact-Theorie.' *Annalen*, Vol. 36, (1840), pp. 161—171. *Ib.* 'Ueber die Gährung und die Quelle der Muskelkraft.'

There was, it will be remarked, a belief in the accidental, the fortuitous, nature of ferment actions which inspired this theory of Liebig's. It partook of the spirit which pervaded theories of generation before the days of Redi and Spallanzani. In a sense the view of Liebig appears even more irrational than that of Berzelius, for it assumes a fortuitous behaviour on the part of bodies whose constancy of behaviour under given conditions is a leading characteristic. Though advanced by one whose extraordinary services in the development of modern chemistry cannot be gainsaid, the theory of Liebig was in opposition to a great number of facts already ascertained at the time when it was promulgated, and the erroneous statements upon which it was based tended more to delay than to further the progress of science.

Whilst the splendid researches of Pasteur[1] at once shewed how far removed are processes of fermentation from the category of fortuitous events, and that every true ferment action which is in any way connected with the changes of a living organism is to be looked upon as the resultant of the chemical activities of that organism. others were proving the groundlessness of other of Liebig's arguments. In a masterly memoir[2], Dumas, *inter alia*, dealt with the physical theory which lay at the very foundation of Liebig's theory, to wit, the possibility of transmitting the state of activity engendered by specific ferments through media which are not pervaded by them. Research soon followed research, which shewed that whilst it is difficult to free the unformed ferments from the proteid bodies which constitute the ground matter of the cell protoplasm in which they are formed, there are but slender grounds for coming to the conclusion that a ferment is essentially a proteid, much less a proteid in a state of decomposition.

Have then the more accurate and correct views of catalytic phenomena to which the progress of science has introduced us enabled us to form any conception as to the way in which an unformed ferment may exert its action? To this question we may reply that amongst the phenomena which used formerly to be explained on the mere hypothesis of 'catalysis,' there are some which are suggestive of the kind of interchanges which probably go on between the above ferments and the bodies of which they effect the decomposition.

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[1] Pasteur. The student will find it most convenient to read the summary of the very numerous researches in this department of knowledge of this great scientific man in his work entitled '*Etudes sur la Bière.*' Paris, Gauthier-Villars, 1876.

[2] Dumas, 'Recherches sur la fermentation alcoolique.' *Comptes Rendus des séances de l'Académie des Sciences*, T. 75, 1872, p. 276.

It appears almost certain that amongst catalytic phenomena, employing the term in the sense in which J. R. Mayer employed it, ferment phenomena resemble those in which there is apparently a periodic synthesis and dissociation of the catalysing agent, which acts in a similar manner to the agent which explodes a train of gunpowder. Amongst chemical phenomena in which one body acts apparently as a go-between, and leads to an almost indefinite series of exchanges of matter and energy, two at once suggest themselves to the mind of the thinker as affording a clue to the probable action of ferments, to wit: (1) the function of hæmoglobin as an oxygen carrier, as a go-between the atmospheric oxygen, on the one hand, and the organic molecules which are oxidised by it in the organism, on the other, and (2) the function of sulphovinic acid in the process of ætherification. In the latter case, however, the results of the process are the reverse of those which follow the normal action of ferments, in so far that whilst the latter as their primary function lead to the decomposition of complex into simpler compounds, the action of sulphovinic acid leads to the synthesis of a more complex out of simpler molecules, to a conversion of kinetic into potential energy.

Enzymes differ in their actions.

The principal enzymes of the alimentary canal belong either to the group of 'proteolytic,' or to that of 'amylolytic' ferments. The enzymes of the first group (pepsin and trypsin), dissolve proteids and effect their more or less profound decomposition. The enzymes of the second class (as the so-called 'ptyalin,' the diastatic enzyme of the salivary glands, and the powerful diastatic enzyme of the pancreas) liquify boiled, gelatinous starch, breaking down the complex starch molecule into molecules of greater and greater simplicity, of which the final representatives are a dextrin, maltose and dextrose.

In addition to the two groups of ferments referred to above, there occur in the alimentary canal 'curdling,' 'inverting,' and 'fat-decomposing' or 'piolytic' ferments. These enzymes will be considered in detail in the sequel.

Circumstances which influence the activity of Enzymes.

i. *Temperature.* All enzymes exert a more energetic action at a moderately high than at a low temperature, though the influence of a rise in temperature is more marked in some cases than in others. ii. *Reaction.* The reaction of the medium in which they are placed, influences remarkably the activity of certain enzymes; thus the proteolytic enzyme of the stomach, *pepsin,* is inactive in neutral or alkaline solutions, the presence of a free acid being essential to its activity; whilst the proteolytic ferment of the pancreas, *trypsin,* acts with feebleness in solutions which are neutral or feebly acid and needs a decidedly alkaline medium for the full exercise of its powers. iii. *Presence* or *absence of excess of certain salts.* The influence exerted by salts upon certain reactions induced by ferments is illustrated, (*a*) by the impossibility of inducing the curdling of casein in the absence of calcium salts. (*b*) by the hindering action exerted by certain neutral salts on the coagulation of the blood, and of certain other salts, as for instance, potassium iodide and bromide on peptic digestion.

In exerting their action are enzymes destroyed?

From a consideration of all the facts bearing on the matter it would appear that in exerting their characteristic actions the various enzymes are in part slowly and gradually destroyed, so that the activity of a given quantity of enzyme cannot be prolonged indefinitely. In most, perhaps in all, cases the accumulation of bodies which result from the ferment action slows and ultimately stops that action long before the enzyme has been exhausted or destroyed, so that, by merely removing the bodies so acting, activity is restored to it. This removal can often be effected by the process of dialysis.

SECT. 1. SALIVA, AND ITS ACTION UPON THE CONSTITUENTS OF FOOD.

INTRODUCTORY SKETCH, CHIEFLY CONCERNING THE SALIVARY GLANDS.

Purposes served by saliva.

The interior of the mouth is continually moistened by a somewhat viscous, tasteless, watery liquid, *the saliva*, a product of the activity of several so-called *salivary glands;* the presence of this liquid facilitates the movements of the tongue, lips and cheeks in articulation. Though essential to proper articulation, the saliva is, however, to be looked upon as one of the digestive juices, and is poured out in much increased quantities when food is introduced into the mouth.

It acts as a solvent of many sapid substances introduced into the mouth, and as the vehicle which brings them into contact with the end organs of the nerves of taste; by moistening the food it renders the essential preliminary act of mastication more easy; it prevents the particles of food from adhering to the interior of the mouth, and thus co-operates with the muscular movements of the lips, tongue, and cheeks in forming the crushed food into a *bolus* which may readily be propelled through the pharynx and œsophagus; lastly, in man and several other animals it exerts, in virtue of the presence of an *enzyme*, which used formerly to be termed ptyalin, and which we now usually term the 'diastatic' or 'amylolytic' ferment of the saliva, a solvent action upon the starchy constituents of food, and thus initiates the chemical operations to which the food is subjected in its progress through the alimentary canal. The saliva exerts, therefore, two sets of functions, the mechanical and the chemical, of which the first are unquestionably the more important, as is shewn by the fact that in many animals the saliva is free from diastatic enzyme and therefore from any chemical activity whatsoever, or contains it in such small quantities that they cannot be supposed to exert any appreciable action.

GENERAL OBSERVATIONS UPON THE STRUCTURE OF THE SALIVARY GLANDS WHEN AT REST.

As has been already said, the saliva is secreted by several glands of which the ducts pour their secretion into the cavity of the mouth, where it is mingled and constitutes the 'mixed saliva.' The chief of these glands are the parotid, submaxillary, and sublingual glands, though their secretion is mixed with that of small glands (*mucous* and *serous*) scattered through the mucous membrane of the mouth and tongue, and which are included under the term of 'buccal' glands. Many animals possess also a fairly large orbital gland, the duct of which opens into the mouth.

Structure of the salivary glands.

The salivary glands all belong to the group of 'acinous' or 'compound racemose' glands, although the terminal alveoli are in reality more tubular than spherical. According to the researches of Heidenhain they may, however, be divided into two groups, which he has denominated *serous*, or albuminous, and *mucous* glands, according to the structure of the cells of their acini, their chemical characters, and the nature of the secretion which they elaborate.

The parotid gland is, in most, if not in all, mammals, an albuminous gland, although a few mucous cells may be present in it. The submaxillary gland is in some animals albuminous, as in the rabbit: in others mucous, as in the dog: in others, again, part albuminous and part mucous, as in man. The sub-lingual gland consists in part of tubes with mucous cells and in part of tubes with albuminous cells; on account of the general preponderance of the mucous element it is classed with mucous glands. The orbital gland is as a rule mucous or serous according as the submaxillary gland is mucous or serous. Glands belonging to the former of these classes secrete a fluid containing some, though it may be only a small, quantity of a proteid coagulable by heat, and resembling, if not identical with, serum-albumin; the mucous glands, on the other hand, as their name implies, secrete a liquid relatively free from albumin; but containing mucin as its characteristic constituent.

In the serous glands hardened in alcohol the epithelium lining the acini is composed of comparatively small, polygonal or rounded cells, of which the outlines are not very distinct until acted upon by certain reagents; the protoplasm, which is but slowly coloured by carmine, presents many dark granules, and the normally spherical nucleus is often shrunken by the reagent.

In the mucous glands the characteristic (mucous) cells of the alveoli are large and clear, very faintly granular, with a rounded or oval nucleus near their periphery surrounded by a little protoplasm. Here too the nucleus may be much shrunken. The part of the cell near the nucleus is usually prolonged into a process which overlaps the neighbouring cell.

In addition to the characteristic mucous cells there are found in the alveoli of some mucous salivary glands when examined in a state of rest, situated at some parts of the periphery, i.e. lying more internal than, or nearer to, the membrana propria than the mucous cells, small cells, possessed of a round nucleus usually aggregated together, and containing much albumin; to these aggregations (first described by *Gianuzzi*[1]) the term demilunes or *lunulæ* has been applied. They may with probability be regarded as albuminous cells which have been overlapped by the larger mucous cells.

Microchemical reactions of the serous and mucous cells.

1. The serous cells possess micro-chemical reactions which indicate the presence within them of proteids; to wit, the addition of dilute mineral acids (containing 0·02 per cent. of acid) causes marked turbidity, an effect which is much more strongly produced by the same acids in a concentrated condition. Acetic acid causes considerable swelling and a clearing up of the cell contents.

2. Mucous cells appear to be composed mainly of mucin or mucigen (?). Their substance is precipitated by acetic acid, whilst it is altogether unacted upon by strong mineral acids.

3. According to Nussbaum[2] the presence of amylolytic ferment within the secreting cells of the salivary glands is rendered evident by the cells assuming a dark colour when brought in contact with a 1 per cent. solution of osmic acid. It has however been shewn by Grützner[3] that the cells of salivary glands, which form no diastatic ferment, as for instance the submaxillary gland of the rabbit, possess the property of reducing osmic acid. Langley[4] has shewn that if a judgment as to the cells which form diastatic ferment were based upon the osmic acid reaction, we should be led to the absurd conclusion that the ferment is chiefly formed by the epithelium lining the ducts and ductlets and the part of the alveolus next to the latter, whilst the alveolar cells proper would enjoy no such function.

Some glands intermediate in structure.

As has been said, in certain mucous glands the mucous cells are supplemented by the cells of the demilunes, though certain mucous glands, as those of the tongue, exist where the typical mucous cells alone occur.

There are glands, and the submaxillary of man is an example, which are termed mixed glands, inasmuch as some of the acini have all the characters of serous, others of mucous glands.

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[1] Gianuzzi, *Ber. d. Sächs. Ges. d. Wiss.*, Sitz. f. 27 Nov. 1867.

[2] Nussbaum, 'Die Ferment-bildung in den Drüsen.' *Archiv f. micros. Anat.* Vol. XIII. (1876), p. 721.

[3] Grützner, 'Ueber Bildung und Ausscheidung von Fermenten.' Pflüger's *Archiv*, Vol. XVI. 1877, p. 105.

[4] Langley, 'Some remarks on the formation of ferment in the submaxillary gland of the rabbit.' *Journal of Physiology*, Vol. I. (1878), p. 68.

The Nervous Supply of the Salivary Glands.

We shall not enter, in this place, into a detailed description of the innervation of any one of the salivary glands, but shall confine ourselves to the following categoric statements.

Each salivary gland is supplied by at least three classes of fibres, viz. secretory fibres, vaso-constrictor and vaso-dilator fibres, of which the first and the third are in general conveyed to the glands in branches of cerebral nerves: these are, the chorda tympani for the submaxillary and sublingual; and the auriculo-temporal (which however derives them through communications with the otic ganglion) for the parotid. The second class, of vaso-constrictor, fibres for the most part run in sympathetic trunks, which, however, also contain secretory fibres.

When therefore one of the cranial branches supplying a gland is stimulated, there occur two acts, viz. secretion[1] and simultaneous dilatation of blood-vessels[2]; that these two acts are not absolutely interdependent is proved by the fact that certain drugs such as atropin[3] paralyse the one set of fibres, leaving the other intact.

When, on the other hand, the sympathetic filaments supplying the gland are stimulated, the blood-vessels of the gland contract, and there is produced a small quantity of saliva differing in physical characters and chemical composition from that obtained under the circumstances first referred to.

Heidenhain's distinction between secretory and trophic nerves.

According to Heidenhain[4], however, in each of the two kinds of nerves supplying a salivary gland there exist, besides the vascular nerve fibres, secretory and trophic, or as we should prefer to term them, '*metabolic*' fibres, though the number of one or other of these classes may be insignificant; the secretory usually predominating in the cranial nerve branches, the trophic in the sympathetic. Stimulation of secretory fibres leads, according to Heidenhain, to an increased flow of water; stimulation of the metabolic to an increased secretion of specific substances, in consequence of the conversion of insoluble into soluble substances, and to an increased production of protoplasm.

There are decided objections to accepting the term *trophic* (which has already been used in a different and fairly well-known sense) to designate those nerve fibres whose action it is specially to increase the metabolism of secreting cells, and we shall therefore in general use *metabolic* in the same sense as Heidenhain's expression trophic. The term *trophic* has been generally employed to designate the action which certain nerve centres exert on the nerve fibres connected with them and on the tissues and organs which are innervated by them. Thus the Gasserian ganglion is said to exert a trophic influence on the eye, as its complete division leads in general to destructive changes in that organ; or to take a case less open to controversy, the ganglion on the posterior root of a spinal nerve is said to exert a trophic influence on the afferent fibres connected with it, because when these are separated from it, and so removed from its influence, they undergo a process of degeneration.

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[1] Ludwig, 'Neue Versuche über die Beihülfe der Nerven zur Speichelabsonderung.' *Zeitschr. f. rat. Mediz.*, N.F. (1851), p. 259.

[2] Claude Bernard, *Comptes Rendus*, 28 Jan. 1858.

[3] Kieuchel, 'Das Atropin u. die Hemmungsnerven.' Dorpat, 1862.

[4] Heidenhain, 'Physiologie der Absonderungsvorgänge.' Hermann's *Handbuch*, Vol. v. p. 55.

Vascular Changes which accompany Secretion.

Ordinarily when a salivary gland passes from the state of rest into that of activity it is at once the seat of an increased blood-flow, which is associated with the dilatation of the blood-vessels of the organ. Under these circumstances the blood leaving the gland presents a florid arterial, instead of a venous colour, which characterises that of the organ when at rest. This vascular dilatation is explained by the coming into action of the before-mentioned vaso-dilator fibres; it is not necessarily dependent on the act of secretion, as it may occur after the secretory fibres have been paralysed.

Heat evolved during Secretion.

As was shewn in a now classical investigation of Ludwig[1], when the salivary glands are thrown into activity there is a rise in temperature, so that the temperature of the saliva leaving the submaxillary gland may exceed by 1° C. that of the blood flowing to the gland. This rise in temperature cannot be explained by a study of the chemical characters of the salivary secretion, but is doubtless the result of the increased metabolic changes which necessarily accompany the act of secretion in the gland cells, and which chiefly affect their protoplasm.

The Secretion of Saliva not an act of filtration.

That the secretion of saliva (and indeed that secretion in general) is not a mere act of filtration, was proved by Carl Ludwig when he shewed that saliva can be secreted by a gland though the pressure exerted by the secreted fluid in the ducts within it is considerably higher than that of the blood circulating through the arteries which supply it. On many grounds it may be positively asserted that the secreting cells are the primary agents in the withdrawal from the blood of the water necessary for the secretion, though the exact nature of the process is yet unknown[2]; similarly on the grounds stated below we know that within the protoplasm of the gland cells the characteristic soluble constituents of the secretion are formed.

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[1] Ludwig u. A. Spiess, 'Vergleichung der Wärme der Unterkiefer-Drüsenspeichel und des gleichzeitigen Carotidenblutes.' *Zeitschr. f. rat. Med.*, 1858, p. 361.

[2] Consult Heidenhain, Hermann's *Handbuch*, Vol. v. p. 72.

Structural Changes in Secretory Cells accompanying activity of the Salivary Glands.

The researches made during the last few years by Heidenhain, Ebstein and Grützner, Langley, and fully confirmed by a large number of observers, have demonstrated that in the salivary glands, as perhaps in all secreting glands, structural and perfectly obvious microscopic changes occur, which stand in close relation to the different conditions of functional activity.

Characters of resting glands.

The resting gland cell is large, but possesses comparatively little protoplasm, and therefore comparatively little matter which can be stained by colouring matters, especially by carmine; it contains, instead, a store of material which has been elaborated in, or at the expense of, the protoplasm. This material does not constitute the specific matter of the secretion, but is its antecedent.

That a chemical difference exists which admits of direct proof is argued by Heidenhain, in the case of the mucous glands for several reasons, but mainly on the ground of the statements of Watney and Klein, that whilst mucin is stained by hæmatoxylin, its antecedent (mucigen) is not affected by that colouring matter. Mr J. N. Langley, who has devoted much attention to this question, has arrived at the conclusion that as yet no evidence whatever exists which warrants the assertion that such a hypothetical *mucigen* exists. In a hitherto unpublished note on the subject which the author has had the advantage of reading, he points out, *inter alia*, that hæmatoxylin when added to saliva does *not* stain the mucin which that fluid contains, *unless* it be in a solution which contains alum.

The behaviour of the gland cells towards hæmatoxylin varies greatly according to the treatment which the tissue has undergone. Langley has frequently obtained sections of gland hardened in alcohol with a stringy mass of mucin in the ducts, and on staining with hæmatoxylin (whether Kleinenberg's, or Böhmer's or Delafield's, or in aqueous solution, or in dilute alkaline solution), obtained good staining of nuclei and demilunes without the mucin having taken a trace of colour. If we are to rely on the hæmatoxylin test we must, in such cases, conclude that the saliva in the duct did not contain mucin but mucigen. 'It is possible,' says Langley, 'that a method of staining with hæmatoxylin may be found which shall give satisfactory results; for sometimes the substance in the duct is stained more than the cells and sometimes the cells are unequally stained, but at present one cannot use hæmatoxylin in such a way as to give such results constantly.' But Langley's criticism is not limited to the chemical grounds which have been alleged in favour of the hypothetical mucigen. He considers that the physiological evidence in favour of its existence is at present insufficient. The further discussion of this question is beyond the scope of the present work.

Characteristics of glands in a state of activity.

When, however, a gland passes into a state of activity, as for example by the irritation of its so-called secretory nerves, the gland cells undergo the following changes, which may proceed simultaneously, though not necessarily so:—the stored-up matter previously referred to is converted into soluble constituents of the secretion, and at the same time there occurs a growth of the protoplasm of the cells, at the expense doubtless of the richer supply of lymph which, during the secretory act, bathes the gland.

The period of activity in so far as the gland cell is concerned is indeed a period of removal of ready-made constituents of secretion; a period in which the protoplasmic constituents of the cells generally increase. According to some, active proliferation of secreting cells occurs; the latter statement is probably incorrect.

Whilst we have in a few sentences sketched the general characters of the changes which glands undergo during secretion, our picture is wanting in all details, and the reader is referred for further information to works on Histology and Physiology[1].

SECT. 2. THE CHEMICAL COMPOSITION OF MIXED SALIVA, OF THE SECRETION OF THE THREE CHIEF SALIVARY GLANDS, AND OF BUCCAL MUCUS.

1. MIXED SALIVA.

Mode of obtaining mixed saliva.

The mixed saliva of man may be obtained in a state of purity, some hours after a meal, by everting the lower lip, depressing the head, and collecting the clear liquid which slowly trickles from the angles of the mouth. An abundant flow of saliva may be provoked by the internal use of Jaborandi or its alkaloid Pilocarpine.

A fairly copious flow may be obtained by inspiring, through the mouth, the vapour of chloroform, or by washing out the mouth with water containing a little ether in solution, or even by chewing a fragment of rhatany root; in the latter case the fluid is naturally mixed with the vegetable fragments, and with the soluble constituents of the drug.

Quantity of saliva secreted by man.

In the case of saliva, as in that of other digestive juices, we possess no mode of determining in a reliable manner the amount of the secretion which is poured out in the physiological condition.

Mitscherlich calculated the probable secretion of mixed saliva to amount to 8 to 10 ozs. daily.

According to Tuczek[2], the salivary glands of adult man secrete during mastication *at the rate of* 1300 grammes of saliva for each 100 grammes of gland-substance, the saliva containing 6·3 grammes of solid constituents, of which 3·9 grammes consist of organic matters.

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[1] Consult specially Heidenhain's systematic account under the heading 'Vorgänge innerhalb der Drüsen während ihrer Thätigkeit' in Hermann's *Handbuch*, Vol. v. Chapter IV. p. 56. The reader will find a more lengthy treatment than is possible in the present work in Dr Michael Foster's *Text-Book of Physiology*, Ed. v.

[2] Tuczek, 'Ueber die von Menschen während des Kauens abgesonderten Speichelmengen.' *Zeitschr. f. Biologie*, Vol. XII. p. 534.

Physical Properties of Mixed Saliva.

Normal saliva is, when perfectly fresh, a clear, transparent, viscid fluid, which on microscopic examination is found to hold in suspension, but very sparsely distributed through it, cells of squamous epithelium which have become detached from the walls of the mouth, besides certain cells denominated *salivary corpuscles*, which are probably leucocytes altered by the action of saliva; these cells, which present some resemblance to leucocytes, are much more globular and contain within their interior granules which exhibit so-called *Brownian* movements in a very remarkable manner.

Specific gravity. The specific gravity of the mixed saliva of man varies between 1·002 and 1·006, the mean being, however, about 1·003.

Reaction. Perfectly normal, human saliva possesses an alkaline reaction, which is least marked after a long fast, and most distinct when the flow of the secretion is at its height. According to Chittenden and Smith the alkalinity corresponds to that of a solution containing 0·08 per cent. of Na_2CO_3[1].

In some persons, especially in the morning, the saliva is found to possess an acid reaction, which is however due to fermentative changes.

Frerichs[2] found that 100 grammes of saliva secreted by himself, during smoking, required 0·150 grammes of sulphuric acid to neutralise it.

THE CHEMICAL CONSTITUENTS OF MIXED SALIVA.

Water. As is indicated by its specific gravity, saliva is a very watery liquid, containing only from five to six parts *per mille* of solid constituents.

The Organic Solids of Saliva.

Proteids and mucin. The solids consist partly of undissolved, suspended, organic matters, especially epithelium, and partly of dissolved organic matters and salts. They always contain a very small quantity of a soluble proteid, which resembles, if it is not identical with, serum-albumin, besides a considerable quantity of mucin.

Diastatic enzyme. In the case of the saliva of man and certain other animals, an enzyme is present which possesses amylolytic properties and exerts a chemical action which appears to be identical with that of diastase. This ferment has frequently been termed *Ptyalin*, though this name was originally applied by Berzelius[1] to the organic matters of the saliva generally, obtained by a method which robbed them of all ferment action, which besides was unknown to the Swedish observer. It is more usual to designate it the Diastatic Ferment (or Enzyme) of the Saliva, or Salivary Diastase. It will be separately discussed in one of the succeeding sections.

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[1] Chittenden and Smith, *Transactions Connecticut Association*, 1885.

[2] Frerichs, Article 'Verdauung,' Wagner's *Handwörterbuch der Physiologie*, Vol. II. Part i. p. 760.

Hüfner's and Munk's discovery of a proteolytic ferment in saliva.

By v. Wittich's method, Hüfner[2] obtained from the salivary glands of the pig a glycerin extract which, in addition to very slight diastatic, possessed feeble but decided, proteolytic activity. This ferment was said to be active in alkaline as well as in acid solutions. Munk[3] obtained a similar ferment from mixed saliva, but found that it was very active in acid solutions.

It is probable that the ferment discovered by both Hüfner and Munk was pepsin, of which minute traces probably make their way into the several fluids of the body. The urine, for instance, is known to contain a trace of diastatic ferment and especially of pepsin, and occasionally, it is said, of trypsin, and of rennet ferment[4].

Extractive matters of saliva.

In disease, certain extractive matters, such as urea, leucine and lactic acid, have been discovered in the saliva. The first of these is probably a normal constituent, though only present in minute traces. We do not yet know what other extractives occur as regular constituents in health.

The Saline Constituents of Saliva.

The Saline Constituents of the Saliva are composed chiefly of alkaline chlorides; they include, however, also alkaline and earthy phosphates, and, in some cases, earthy carbonates. They are distinguished by the presence of a salt whose formation appears characteristic of the salivary glands, viz., a soluble sulphocyanate.

Discovery of a sulphocyanate in saliva.

Treviranus[5] was the first to observe that when a solution of ferric chloride is added to saliva it produces a reddish colour, which was subsequently conclusively shewn by Tiedemann and Gmelin[6] to be due to the presence of sulphocyanic acid in saliva.

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[1] Berzelius, *Traité de Chimie*. Nouvelle édition par Valerius (1839), Vol. III. p. 591.

[2] Hüfner, 'Untersuchungen über die ungeformten Fermente.' *Journ. f. prakt. Chemie*, New Ser. Vol. 5, p. 372.

[3] Munk, 'Untersuchungen über die ungeformten Fermente im Thierkörper.' In Maly's *Jahresbericht*, Vol. 6, p. 270.

[4] W. Sahli, 'Ueber das Vorkommen von Pepsin und Trypsin im normalen menschlichen Harn,' Pflüger's *Archiv*, Vol. 36 (1885), p. 209. A. Stadelmann, 'Ueber Fermente im normalen Harn,' *Zeitschrift f. Biologie*, Vol. 24 (1888), p. 226; also 'Untersuchungen über den Pepsin-Ferment-gehalt des normalen und pathologischen Harnes.' *Zeitschrift f. Biologie*, Vol. 25 (1889), p. 215.

[5] Treviranus, *Biologie*, 1814, Vol. IV. p. 330.

[6] F. Tiedemann und L. Gmelin, *Die Verdauung nach Versuchen*. Heidelberg u. Leipzig, 1826, Vol. I. p. 8, et seq.

Mode of demonstrating presence of a sulphocyanate in saliva.

(*a*) Human saliva is treated with a small volume of a pale and acidulated solution of ferric chloride. A reddish colouration of varying intensity, but tending to that of diluted claret, is generally produced. This colour is bleached by the subsequent addition of a solution of corrosive sublimate.

(*b*) According to Gscheidlen's[1] method, filter-paper is dipped in a weak solution of ferric chloride containing some free hydrochloric acid and then allowed to dry. The contact of a drop of saliva with such paper occasions a reddish stain. The author can strongly recommend this method.

(*c*) *Solera's Reaction.* When human saliva is treated with a solution of iodic acid it assumes a yellowish colour, due, according to Solera[2], only to the sulphocyanate present, which liberates iodine; the latter is subsequently easily detected by the addition of starch. This reaction is said to permit of the detection of 0·00000004 grm. of a sulphocyanate.

Is a sulphocyanate constantly present in saliva?

A sulphocyanate is not constantly present even in human saliva. According to Hoppe-Seyler, with whose experience on this matter that of the author does not coincide, it is indeed frequently absent[3]. In the saliva of the dog Hoppe-Seyler has never discovered a sulphocyanate, whilst both Schiff and Solera discovered it in the mixed saliva and in the secretion of the parotid gland.

It has been asserted by Schiff that the quantity of a sulphocyanate, as determined by the depth of tint produced by the addition of an iron salt, increases after saliva has been secreted, but the statement appears to be inaccurate.

Proportion of sulphocyanic acid in saliva.

According to Munk[4], whose method of quantitative analysis will be described in a subsequent section of this chapter, the proportion of sulphocyanic acid in mixed human saliva amounts to 0·01 per cent.

Constitution and probable origin of sulphocyanic acid.

Sulphocyanic acid $\left.\begin{matrix}CN\\H\end{matrix}\right\}S$ has a constitution exactly analogous to that of cyanic acid $\left.\begin{matrix}CN\\H\end{matrix}\right\}O$, sulphur occupying the position of the oxygen of the latter body.

In the metabolic decomposition of the proteids the chief nitrogenous product is carbamide or urea, a body which can be formed with remarkable ease by a rearrangement of the atoms of ammonium cyanate, and whose formation probably points to the presence within the proteid, at least at the time of decomposition, of cyanogen residues.

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[1] Gscheidlen, 'Rhodannachweis' Privatmittheilung. Maly's *Jahresbericht*, Vol. IV. p. 91; also consult Gscheidlen, 'Ueber das constante Vorkommen einer Schwefelcyanverbindung im Harn der Säugethiere.' Pflüger's *Archiv*, Vol. 14 (1877), p. 403.

[2] Solera, 'Di una particolare reazione della saliva' abstracted in Maly's *Jahresbericht*, Vol. VII. p. 256. 'Indagini sulle manifestazioni objettive del solfocyanuro potassico salivare.' Pavia, 1877. Abstracted in Maly's *Jahresbericht*, Vol. VIII. p. 235.

[3] Hoppe-Seyler, 'Bei weitem nicht alle Menschen haben schwefelcyanhaltigen Speichel.' *Physiologische Chemie*, Part II. p. 186.

[4] J. Munk, 'Schwefelcyanbestimmung im Speichel.' Virchow's *Archiv*, Vol. LXIX. p. 350.

The sulphocyanates of the saliva are doubtless likewise derived from the decomposition of proteids. Inasmuch as ammonium sulphocyanate can be transformed into sulphur-urea, just as ammonium cyanate can be transformed into urea proper, it would appear probable that some sulphur-urea should occur in the organism. Maly made a search for this body, but in vain.

Sulphocyanic acid was found by Leared[1] to be an almost constant constituent, not only of the saliva but also of the blood and urine of man and certain other animals, and his observations have been confirmed by those of Gscheidlen[2] and Munk[3]. In the urine, Leared found one half grain in 16 ozs., or 0·11 grm. per litre. Gscheidlen estimated the amount at 0·02 grm., and Munk at 0·08 grm. per litre. Gscheidlen believes that the sulphocyanic acid of urine is derived from the blood, into which it passes, by absorption from the saliva by the alimentary canal, for when he diverted the secretion from the alimentary canal by establishing salivary fistulæ he failed to detect a sulphocyanide in the blood and urine.

Dr Fenwick[4], who has paid much attention to the variation in the amount of sulphocyanic acid in the saliva, believes that the salt is a product, and so indirectly affords evidence, of the nitrogenous metabolism of the organism, and that its amount diminishes, under conditions in which the activity of the nutritive functions is diminished. He considers, though on evidence which is indirect and unsatisfactory, that the sulphocyanic acid of the saliva is genetically related to the organic sulphur compound of the bile, an opinion which he bases especially on the statement that whenever the bile is prevented from reaching the alimentary canal, the sulphocyanide of the saliva disappears. It appears desirable that the statement of Fenwick should be controlled by observations on animals in which biliary fistulæ have been successfully established.

Presence of ammonia in saliva.

By the direct addition of Nessler's reagent, traces of ammonia can readily be detected in saliva[5].

Presence of nitrites in saliva.

According to Peter Griess[6] saliva always contains nitrites which may be detected by means of a solution of meta-diamidobenzol.

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[1] Leared, 'On the presence of sulphocyanides in the blood and urine.' *Proceedings of the Royal Society*, Vol. 18 (1870), p. 16.

[2] Gscheidlen, 'Ueber das constante Vorkommen einer Schwefelcyanverbindung im Harn der Säugethiere.' Pflüger's *Archiv*, Vol. 14 (1877), pp. 401—412.

[3] T. Munk, 'Ueber das Vorkommen von Sulfocyansäure im Harn und ihre quantitativen Verhältnisse.' Virchow's *Archiv*.

[4] Fenwick, *The Saliva as a test for functional Disorders of the Liver*. 8vo. London, 1889.

[5] Maly, Hermann's *Handbuch*, Vol. v. Part ii. p. 8.

[6] P. Griess, *Ber. d. d. chem. Gesell.*, Vol. II. p. 624.

The saliva to be tested is diluted with five times its volume of water, acidulated with sulphuric acid, and then treated with a solution of the reagent. An intense yellow colour is produced. Griess has by this colorimetric method estimated the nitrous acid to amount to between 1 and 10 milligrammes per litre of saliva.

The gases of mixed saliva.

The gases of mixed saliva have not been investigated, and from the nature of the fluid they doubtless will vary considerably. We may however conclude that they will not sensibly differ in composition from those which may be obtained from the submaxillary saliva.

Results of Quantitative Analyses of Mixed Human Saliva.

The following analyses exhibit the results obtained by Frerichs, Jacubowitsch, and Herter[1]:

	(1) Frerichs.	(2) Jacubowitsch.	(3) Herter.
Water in 1000 pts.	994·10	995·16	994·698
Solids	5·90	4·84	5·302
Soluble organic matters ...	1·42	1·34	3·271
Epithelium	2·13	1·62	?
Potassium Sulpho-cyanate	0·10	0·06	?
Inorganic Salts..............	2·19	1·82	1·031.

The following are the results of analyses of the ash of Saliva.

Jacubowitsch. In 1000 parts of Saliva.	(1) Man.	(2) Dog.
Total Salts	1·82	6·79
Phosphoric Acid......	0·51	0·82
Sodium	0·43	
Lime	0·03	0·15
Magnesia	0·01	
Alkaline Chlorides...	0·84	5·82

Enderlin. Human Saliva. In 100 parts of the Ash.	
Soluble Salts	92·367
Insoluble „	5·509
Alkaline Chlorides ...	61·930
Sodium Phosphates ...	28·122
„ Sulphate	2·315

2. PAROTID SALIVA.

Introductory Observations.

The Parotid gland, as has already been stated, is the type of a so-called serous salivary gland, that is, of a gland whose secretory cells are comparatively rich in proteid matters, and which secretes a watery liquid, destitute, or *nearly so*, of mucin, and containing a trace of a soluble albuminous substance in solution.

The secretion of parotid saliva is unquestionably related in a peculiarly close manner to the mechanical movements of mastication, as is proved by many facts. In animals with parotid fistulæ, the secretion will be observed to flow whenever the muscles of mastication come into action, and the quantity secreted is closely related to the degree of activity of these muscles. Thus if cannulæ be tied into the Stenonian ducts of a ruminant, it will be observed that during mastication of the food, which in these animals is effected alternately by the molars of the two sides of the jaws, the flow of parotid saliva alternates likewise, being always more abundant on the side on which the muscles of mastication are more active. Again, if the amount of parotid saliva flowing from a parotid fistula be observed in the case of a horse which is fed first upon dry aliments such as oats, requiring powerful efforts of mastication, and then upon soft watery food such as bran mash, the amount of the salivary fluid secreted in the first case will be very much greater than in the second.

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[1] Hoppe-Seyler, *Physiologische Chemie*, p. 188.

These facts, which we derive from the researches of Claude Bernard, establish clearly enough the peculiarly close relationship which exists between the parotid secretion and the mechanical movements of mastication, a relationship the existence of which is also borne out by the fact that the parotid is relatively more highly developed in animals, such as ruminants, in which the process of mastication is most perfectly performed, and less developed in animals, like carnivores, in which the food is swallowed in large masses. In intimate agreement with the part which it has to play in moistening the aliments which are to be subjected to the process of mastication, we find the parotid saliva to be less viscid and more watery than the secretion of the other salivary glands.

The nerves which influence the parotid secretion.

The nerve fibres which influence the secretion of parotid saliva are derived (*a*) from the sympathetic, (*b*) from the glosso-pharyngeal nerve, though the course which the latter pursue is one peculiarly devious, viz. through its tympanic branch or nerve of Jacobson, to the small superficial petrosal, thence to the otic ganglion[1], and from it into the auriculo-temporal nerve, of which branches pass into the parotid gland. According to the view of Heidenhain, the *secretory* fibres, i.e. those which influence the quantity of the secretion by particularly influencing the amount of water which the gland separates from the blood, are derived from the glosso-pharyngeal, whilst the trophic, which directly influence the metabolism of the secretory cells, are contained in the sympathetic filaments distributed to the gland.

A stimulation of Jacobson's nerve causes in the dog an abundant flow of watery saliva; a stimulation of the sympathetic does not apparently in the dog occasion any secretion, but it increases very notably the amount of solid matter contained in the secretion which flows on simultaneous stimulation of Jacobson's nerve. In the cat and rabbit stimulation of the sympathetic causes secretion, very much as in the case of the submaxillary gland[1].

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[1] Doubts long prevailed as to the source of the secretory fibres which reach the parotid through the otic ganglion. The opinion long prevailed that these were derived through the *small superficial petrosal* n. from the 7th, but the careful experiments of Eckhard and Heidenhain have shewn that whilst intracranial stimulation of the 7th is never followed by a flow of parotid saliva, this result is always obtained when Jacobson's nerve is stimulated. We must therefore perforce abandon the seductive theory that the 7th nerve supplies the secretory filaments to all the salivary glands, and admit that the mainly sensory glosso-pharyngeal shares these functions.

Mode of obtaining pure parotid saliva.

In man the parotid saliva may be collected by inserting a tube through the mouth into the duct of Steno[2]. In the lower animals by exposing the duct and tying a silver cannula into the duct.

Insertion of a cannula into the human parotid duct.

"As it is hardly possible to insert a cannula into one's own parotid duct, a second person must be employed, who should sit opposite a good light. . . . . The method is as follows: Draw one angle of the mouth outwards and forwards so as to stretch the cheek. Opposite the second molar tooth of the upper jaw the small papilla is seen which marks the orifice of Stenson's duct. Insert the cannula and hold it steadily but carefully in its place, then a third person may blow into the mouth some vapour of ether, or introduce a little diluted tincture of pyrethrum[3]."

Insertion of a cannula into the parotid duct of the dog.

In a deeply anæsthetized animal, the hair is clipped "from the cheek between the orbit and the angle of the mouth. On running the finger along the lower border of the zygomatic arch from behind forwards, its anterior and inferior root is felt at its insertion into the superior maxilla, forming an arch of which the convexity is directed backwards. At the end of this arch, between its insertion into the maxillary bone and the alveolus of the second molar tooth, a little depression is felt. Exactly on a level with this depression, and in a line with the insertion of the zygomatic arch, make an incision through the skin, cutting obliquely in a direction from the inner canthus of the eye towards the angle of the mouth.

"On dividing the subcutaneous cellular tissue, the facial vein and artery, a nerve and the parotid duct will be found all together. The duct lies more deeply and runs from behind forwards, while the artery with its accompanying vein pass from above downward.

"It is of a pearly white colour. Isolate it and divide it as near the mouth as possible. The wound must be closed round the duct, and the duct secured in it by a suture, just as in the case of the submaxillary gland[4]."

Physical and Chemical Characters of Normal Parotid Saliva.

Physical characters of parotid saliva.

Parotid saliva of man is a clear, transparent, non-viscid liquid, possessed of an alkaline reaction[5], except at the very commencement of its flow, when its reaction may be faintly acid. Its specific gravity varies between 1003 and 1006, or according to Hoppe-Seyler between 1006·1 and 1008·8. It contains no formed elements.

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[1] Langley found (*Journal of Physiology*, Vol. x. 1889, p. 320) that for a time after the parotid of the dog has been secreting, either reflexly or by stimulation of Jacobson's nerve, stimulation of the sympathetic *always* produced a secretion. Similarly when a secretion is going on after injecting pilocarpin, stimulation of sympathetic increases at first the rate of secretion and then slows it. So the sympathetic in the dog as in other animals contains secretory fibres, the ordinary decrease of secretion on stimulating the sympathetic being possibly due to the sympathetic vaso-constrictor fibres acting more promptly and completely in the dog's parotid than elsewhere.

[2] Eckhard, *Beitr. z. Anat. u. Physiol.*, Vol. II. p. 205.

[3] Brunton, *Handbook of the Physiological Laboratory*, p. 467.

[4] *Ibid.* p. 468.

[5] See abstract of papers on the reaction of saliva by Astaschewsky and Fubini in Maly's *Jahresbericht*, Vol. VIII. (1878), pp. 234 and 235.

Chemical characters. Parotid saliva contains as a rule no mucin, but small quantities of soluble albuminous bodies; it is therefore partly coagulable by heat and by mineral acids. In the dog

TABULAR VIEW EXHIBITING THE RESULTS OF SOME OF THE CHIEF ANALYSES OF PAROTID SALIVA[1].

	Human.		From Dog.		From Horse.	From Cow.
	I. Mitscherlich.	II. Hoppe-Seyler.	III. Schmidt and Jacubowitsch.	IV. Herter.	V. Lehmann.	VI. Laissaigne.
Water	985·4 to 983·7	993·16	995·3	993·8—991·5	990·0	990·7
Solid Constituents	14·6 to 16·3	6·84	4·7	6·1— 8·47	10·0	9·3
Organic Matters	9·0	3·44	1·4	1·53	2·06—6·0	
KSCN	0·3	—	—	—	—	—
KCl			2·1	6·251		
NaCl	5·0	3·40			4·8 —8·73	
$CaCO_3$			1·2	0·688		

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[1] Compiled from Hoppe-Seyler, *Physiologische Chemie*, pp. 198 and 199, and Maly (Hermann's *Handbuch*, Vol. v. part ii. pp. 16 and 17).

it sometimes, however, contains mucin. According to Kühne there is evidence that the proteids consist of a globulin-like body, of an alkaline albuminate and albumin[1].

The parotid saliva of the lower animals usually gives with ferric chloride a white precipitate containing proteids; that of man on the other hand usually displays the sulphocyanide reaction.

Parotid saliva when exposed to the air either deposits a slight sediment of calcium carbonate, or becomes covered with a pellicle consisting of amorphous or crystalline calcium carbonate.

Composition of parotid saliva obtained by stimulation of sympathetic and glosso-pharyngeal contrasted.

Stimulation of Jacobson's nerve (tympanic branch of glossopharyngeal) as already stated at p. 22, occasions a flow of watery saliva rich in salts and poor in proteids. In the majority of cases stimulation of the sympathetic fibres going to the parotid in the dog does not produce any flow of saliva; if, however, this stimulation be superadded to that of Jacobson's nerve, there is a marked increase in the amount of solid matter and especially of the organic solid matter excreted[2]. This statement rests upon such observations as those of which the results are subjoined.

COMPOSITION OF PAROTID SALIVA OBTAINED BY STIMULATION OF JACOBSON'S NERVE WITH AND WITHOUT SIMULTANEOUS STIMULATION OF THE SYMPATHETIC. (HEIDENHAIN[3].)

		In 100 parts.		
		Total solids.	Salts.	Organic solids.
1.	Stimulation of Jacobson's nerve *without* stimulation of sympathetic	0·56	0·31	0·24
2.	" " *with*	2·42	0·36	2·06
3.	" " *without*	1·03	0·26	0·76
4.	" " *with*	1·74	0·32	1·41
5.	" " *without*	0·57	0·36	0·21
6.	" " *with*	0·64	0·25	0·38
7.	" " *without*	0·49	0·32	0·16

3. SUBMAXILLARY SALIVA.

Introductory Observations.

The submaxillary gland in most animals is typically a *mucous* gland, and its secretion is characterized by viscidity due to the presence of mucin.

The submaxillary gland is relatively more highly developed in carnivorous than herbivorous animals. Claude Bernard sought to establish the specially close connection between the submaxillary saliva and gustation, and called attention to the fact that whilst the parotid enters into activity during the mechanical movements of mastication, the submaxillary gland is not affected by them; whilst on the other hand the stimulation of the sensory nerves of the mouth or, in some cases, the anticipation of food lead to a flow of submaxillary saliva. There can be no question, however, that the parotid likewise is affected reflexly by stimulation of the nerves of taste and directly by ideas associated with food.

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[1] Kühne, *Lehrbuch*, p. 14.

[2] Heidenhain, 'Morphologische Veränderungen der Drüsen während der Thätigkeit,' Hermann's *Handbuch*, Vol. v. (1880), Part i. p. 61.

[3] Heidenhain, 'Beziehungen der Halssympathicus zur Parotis beim Hunde.' Hermann's *Handbuch*, Vol. v. pp. 54 and 55.

The nerves which influence the submaxillary secretion.

The cerebral fibres for the submaxillary gland are contained in the chorda tympani, a branch of the facial nerve. It also receives fibres from the sympathetic. The former according to Heidenhain's theory are mainly secretory, and the latter mainly trophic (metabolic), but there is some doubt (Langley) whether the difference observed between them may not be due to the concurrent difference in the blood supply.

Mode of obtaining submaxillary saliva.

In man, the submaxillary saliva may be collected by introducing a silver cannula into the entrance of Wharton's duct in the mouth; in the lower animals, especially the dog, by cutting down upon the duct and tying a silver cannula into it.

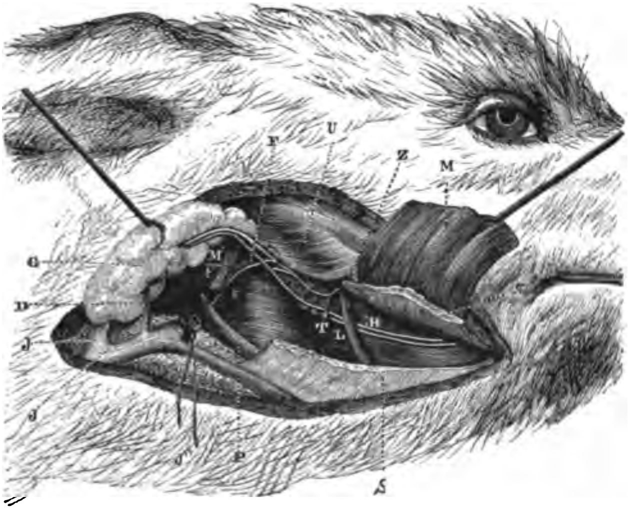

Fig. 1. *M*, anterior portion of the digastric muscle, of which the posterior portion has been removed, and the attachment to the temporal bone cut through at *M'*. *G*, submaxillary gland raised by means of a hook so as to shew its deep surface. *H*, ducts of the submaxillary and sublingual ducts; the former may be traced back to its gland. *J*, trunk of the external jugular vein. *J'*, a branch of the external jugular vein passing to the back of the gland. *J''*, a branch of the jugular passing to the front of the gland cut across. *D*, a venous trunk issuing from the submaxillary gland and joining the external jugular vein. *t*, *t'*, external carotid artery accompanied by two filaments of the sympathetic nerve. *F*, origin of the inferior artery of the gland. *P*, hypo-

glossal nerve. *L*, gustatory or lingual nerve, bridging over the salivary ducts *H*. On raising the inner border of the divided mylo-hyoid muscles *S*, *S'*, the lingual nerve may be traced up and the chorda tympani nerve, *T*, may be seen passing away from the lingual forming a curve, of which the convexity looks downwards. The nerve is seen running towards the hilus of the gland, following a parallel course above the submaxillary duct. *U*, masseter muscle. *Z*, point of origin of the mylo-hyoid nerve, of which the filaments are hidden by the notched digastric and mylo-hyoid muscles.

Physical and Chemical Characters of Normal Submaxillary Saliva.

The submaxillary saliva of man.

Like the gland which secretes it, the saliva of the submaxillary gland is characterized by the presence of mucin, which imparts to it some viscosity. In man this saliva is more fluid than in the dog; it acquires increased viscidity some time after its secretion. It possesses an alkaline reaction, and has a specific gravity which varies between 1002 and 1003; it contains from 3 to 4 parts per 1000 of solid matters, of which the most abundant is mucin, though traces of proteids and diastatic ferment are also present. In reference to the latter it may be said that in most animals whose saliva possesses diastatic properties the submaxillary saliva is more active than the parotid.

When exposed to air it deposits flocculi. According to Eckhard it contains no sulphocyanic acid, but recent authors agree in stating that though this constituent is present in much smaller quantities than in the secretion of the parotid it is not altogether absent[1].

Submaxillary saliva of the dog.

In the dog the submaxillary saliva has been subjected to very thorough investigation under the most diverse circumstances. The secretion is in this animal more viscid than in man; it does not contain diastatic ferment.

The results of quantitative analyses.

The most recent and most complete analyses of the normal submaxillary saliva of the dog have been performed by Herter[2], and the results are stated in the following table.

COMPOSITION OF NORMAL SUBMAXILLARY SALIVA OF THE DOG.
(HERTER.)

	I.	II.	III.	IV.
Water	994·385	994·969	995·411	991·319
Solid matters	5·615	5·031	4·589	8·681
Organic matters	1·755			
Mucin contained in o.m.	0·662			2·604
Inorganic salts, soluble	3·597			5·209
,, insoluble	0·263			1·123
CO_2 in a state of chemical combination	0·440	0·504	0·654	

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[1] Consult Maly, 'Chemie der Verdauungssäfte und Verdauung.' Hermann's *Handbuch*, Vol. v. p. 18.

[2] Hoppe-Seyler's *Physiologische Chemie*, Part II. p. 191.

Analyses I. and III. were of saliva obtained by stimulating the mouth with vinegar; II. of saliva which flowed spontaneously immediately after the fistula had been established, and IV. of saliva poured out during mastication.

Inorganic salts of submaxillary saliva.

The soluble salts consist mainly of sodium chloride and sodium carbonate; the insoluble, of calcium carbonate and phosphate.

Characters of Submaxillary Saliva secreted on stimulation of the Chorda Tympani.

When the chorda tympani is stimulated there is obtained an abundant flow of submaxillary saliva possessing its normal fluidity. At the same time there occur changes in the circulation in the gland which do not specially concern us, and a rise in temperature which may according to Ludwig's determination amount to 1°·5 C. This secretion on stimulation of the chorda may be provoked even when the circulation has ceased; for example, in the gland of a decapitated head. Heidenhain has shewn that, although 'chorda-saliva' is, as was before known, poor in organic solids as compared with the saliva which flows on stimulation of the sympathetic filaments, yet the secretion varies with the intensity of the stimulus.

Influence of intensity of stimulus on amount of secretion and on organic and saline constituents.

The amount of saliva secreted augments perceptibly as the stimulation of the chorda increases in intensity.

At the same time, the salts of the saliva increase very materially, until their proportion attains 0·5—0·6 p. c. The proportion of salts always increases with the proportion of water secreted. This result is obtained whatever the time at which the stimulation has been commenced, even if secretion has been going on for hours.

Langley and Fletcher from an extended investigation on the subject have arrived at the conclusion that '*the secretion of organic substance depends wholly, or almost wholly, upon the strength of the stimulus, whilst the secretion of water and of salts depends also upon the amount of blood flowing through the gland*[1].'

As the amount of organic constituents secreted in the saliva depends intimately upon the store of matters which the secreting cells contain, it follows that the amount of organic matters secreted upon stimulation will depend very greatly upon the work which the gland has previously done.

If the chorda tympani be stimulated when the submaxillary gland has been in repose, it is noticed that on increasing the strength of the stimulus there is increase not only in the amount of the secretion poured out in one minute, and in the amount of total solids and salts, but also of the organic matters; if the gland is however exhausted (compare stimulations 5 and 6, 7 and 8, in the table appended below), then, in spite of increased strength of stimulus, the organic matters will diminish, the influence of fatigue making itself more obvious than that of the increased intensity of stimulus.

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[1] J. N. Langley and H. M. Fletcher, 'On the Secretion of Saliva, chiefly on the Secretion of Salts in it.' *Phil. Transact.* Vol. 180 (1889), B, p. 132.

RESULTS OF AN EXPERIMENT IN WHICH THE CHORDA TYMPANI WAS SUBJECTED TO ELECTRICAL STIMULATION OF VARYING INTENSITY. (HEIDENHAIN[1].)

Number of the stimulation.	Time of duration.	Distance of secondary from primary coil of induction coil.	Total quantity of saliva secreted.	Quantity of saliva secreted in 1 minute.	Proportion of solid matters per cent.	Proportion of salts per cent.	Proportion of organic matters per cent.
	H. M. H. M.						
1	9. 17—9. 38	325—265	3·6 c.c.	0·18 c.c.	1·45	0·29	1·15
2	9. 41—9. 43	220—210	4·4 "	2·2 "	2·28	0·44	1·84
3	9. 56—10. 16	315—295	4·4 "	0·22 "	1·91	0·32	1·59
4	10. 18—10. 20	100—80	4·0 "	2·00 "	2·67	0·58	2·09
5	10. 30—10. 53	320—290	3·6 "	0·15 "	2·22	0·34	1·85
6	11. 1—11. 2¼	200—180	4·0 "	3·20 "	1·88	0·58	1·29
7	11. 12—11. 30	315—295	3·5 "	0·19 "	1·23	0·25	0·98
8	11. 32—11. 35	240—200	5·0 "	1·60 "	1·24	0·37	0·86
9	11. 37—11. 39	100—50	5·0 "	2·50 "	1·88	0·57	1·30

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[1] Heidenhain, Pflüger's *Archiv*, Vol. XVII. (1878), p. 7, and Hermann's *Handbuch*, Vol. V. Part i. p. 52. (*Physiologie der Absonderungsvorgänge.*)

The above most interesting facts are explained by the hypothesis of Heidenhain, that the chorda tympani contains both secretory fibres—that is fibres which influence the proportion of water and of salts secreted—and trophic (metabolic) fibres, which influence the discharge of organic constituents from the secreting cell; further, that the latter set of fibres require a more powerful stimulus to excite them than the former; and lastly, that even though the stimulus be strong, if there be not a supply of available matter in the gland cell the stimulation of the metabolic fibres must remain without effect.

The influence of fatigue in decreasing the solids, but particularly the organic solids, discharged by the submaxillary gland, on stimulation of the chorda tympani, had already been shewn very conclusively by the much earlier experiments by Ludwig and Becker[1], as may be seen by glancing at the subjoined statement in which the numbers 1, 2, 3 and 4 in the first column indicate the order in which the samples were obtained.

TABLE SHEWING DECREASE OF SOLIDS IN SUBMAXILLARY SALIVA OF DOG ON CONTINUED STIMULATION OF THE CHORDA TYMPANI. (LUDWIG AND BECKER.)

Number of sample.	Quantity of saliva in grammes.	Organic residue.	Inorganic residue.	Total residue.
1	5·188	1·12	0·61	1·73
2	13·812	1·07	0·61	1·68
3	11·744	·93	0·67	1·62
4	17·812	·58	0·64	1·22

In the above experiment a decrease of the organic matters of the saliva under continued stimulation is observed; in a subsequent experiment by the same authors, it was found that the mineral or inorganic constituents of the saliva exhibited almost as remarkable a diminution, falling from 0·75 per cent. to 0·48.

Characters of submaxillary saliva secreted on stimulation of the Cervical Sympathetic.

Stimulation of the cervical sympathetic, or of the filaments proceeding from this nerve to the submaxillary gland, leads in the dog to a very scanty secretion of alkaline, very viscid and extremely thick saliva, containing a large quantity of solid matter, chiefly mucin. Whilst the specific gravity of submaxillary saliva of the dog, secreted under the influence of stimulation of the lingual nerve, was found by Eckhard to be 1004·6, that flowing on stimulation of the sympathetic had a specific gravity of 1015·6. The amount of solid matter in the sympathetic saliva of the dog was found by Eckhard[1] to be 2·7 per cent., by Heidenhain sometimes to be as much as 3·74 and 5·86 per cent. On long-continued stimulation, Heidenhain found that the solid matters decreased so that the saliva[2] acquired the characters of that secreted under the influence of stimulation of the chorda tympani. This is in accordance with the facts given above with regard to the gradual exhaustion of the mesostate of the gland-cells.

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[1] Ludwig and Becker, *Zeitschrift f. rat. Med.* N. F. 1 (1851), p. 278.

Differences in effect of stimulating nerves in Cat and Dog.

Langley has shewn that the effects of stimulating the chorda tympani and sympathetic of the cat are different from those observed in the case of the dog. In the cat, sympathetic saliva is in most cases less viscid than the chorda saliva. Moreover whilst atropin paralyses the chorda secretion in the dog, leaving the sympathetic secretion unaltered, in the cat it paralyses the sympathetic secretion as well as that of the chorda[3]. From this it would appear that relatively more secretory fibres run by the sympathetic in the cat than in the dog.

The following analyses shew the usual difference in percentage composition between chorda- and sympathetic-saliva in the cat (Langley[4]).

	Percentage of organic substances.	Percentage of ash.	Total Percentage of solids.
I.			
(1) Saliva obtained by weak stimulation of the left sympathetic	0·353	0·442	0·795
(2) 5 mgrm. atropin given; saliva obtained by strong stimulation of right sympathetic	0·525	0·454	0·979
II.			
(1) Saliva obtained by stimulating chorda, shocks fairly felt by tip of tongue	0·865	0·339	1·205
(2) Saliva obtained by somewhat stronger stimulation of sympathetic	0·426	0·275	0·701

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[1] Eckhard, 'Ueber die Unterschiede des Trigeminus- und Sympathicusspeichels der Unterkieferdrüse des Hundes.' *Beiträge zur Anat. u. Phys.*, Vol. II. p. 205.

[2] Heidenhain, 'Physiologie der Absonderungsvorgänge.' Hermann's *Handbuch*, Vol. v. Part i. p. 48.

[3] Langley, 'On the Physiology of the Salivary Secretion. Part I. The influence of the Chorda Tympani and Sympathetic Nerves upon the secretion of the sub-maxillary gland of the Cat.' *Journal of Physiology*, Vol. I. (1878), p. 96 *et seq.*

[4] Langley, 'On the Physiology of the Salivary Secretion.' Part III. *Journal of Physiology*, Vol. VI. p. 92.

Characters of so-called 'Paralytic' Submaxillary Saliva.—'Antilytic' secretion.

It was pointed out by Claude Bernard[1] that after the nervous influence had been cut off from the submaxillary gland, a very watery saliva is continuously poured out. This so-called 'paralytic' secretion occurs if the chorda tympani alone has been divided.

Heidenhain[2] in a most interesting investigation on this subject discovered the very remarkable fact that when the chorda tympani is divided there follows a continuous secretion from both submaxillary glands, although the secretion is more abundant on the side on which the section has been made. Whilst the term 'paralytic' may be applied to the secretion flowing from the gland whose connexion with the nerve centres has been severed, we may in accordance with Langley's suggestion[3] call the secretion which is set up on the opposite side of the body the *anti-paralytic* or '*antilytic*' secretion.

Langley's researches, which have corroborated and extended our knowledge of the facts discovered by Heidenhain, seem to render it certain that the flow of saliva—both paralytic and antilytic—which follows section of the chorda tympani on one side, is primarily due to a change set up in the secretory centres which are situated in the brain and which result in a continuous stimulation of both glands, the stimuli, in the case of the paralytic flow, travelling along sympathetic fibres, whilst in the case of the antilytic secretion they presumably pursue both courses open to them. The change in the nerve centres brought about by section of the chorda appears to be of the nature of an increase of their excitability; this is supported by the fact that in the altered condition induced by nerve section, a state of dyspnœa induces an increase in the flow of saliva not observed when the nerves are intact.

The fact, however, that a paralytic secretion persists even after the submaxillary gland has been entirely severed from its connection with the central nervous system negatives the idea that the secretion is entirely dependent upon it, and the hypothesis which has been framed in order to account for the facts is that the paralytic secretion, in its later stages, depends upon an excitation of the local nerve centres situated in the gland itself.

The hypothesis is supported by the fact discovered by Vulpian[4] that pilocarpin causes a secretion from the submaxillary gland fourteen days after section of the chorda and sympathetic nerves, as well as that apnœa, dyspnœa and anæsthetics affect the secretion in a manner which is only intelligible by assuming that these act through a nerve centre.

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[1] Bernard, *Journal de l'Anatomie et de la Physiologie*, Vol. I. (1864), p. 507.

[2] Heidenhain, 'Beiträge zur Lehre von der Speichelsecretion.' *Studien des phys. Instituts zu Breslau*, Heft IV. (1868), p. 73.

[3] J. N. Langley, 'On the Physiology of the Salivary Secretion.' Part III. 'The Paralytic Secretion of Saliva.' *Journal of Physiology*, Vol. VI. p. 71.

[4] Vulpian, *Comptes Rendus*, T. 87 (1878), p. 350.

Effect of injecting dilute salt solution into the blood.

Langley has found that 'injection of dilute salt solution in moderate quantity increases the rate of secretion of saliva with a given stimulus, the percentage of salts in the saliva rising nearly normally; and that injection of dilute salt solution in larger quantity increases further the salivary secretion with a given stimulus, but in this saliva the percentage of salts rises much less than normally, and may even fall[1].'

Effect of injecting into the blood a 2 per cent. solution of Na_2CO_3.

Langley investigating the action produced when a weak solution of sodium carbonate is introduced into the blood, arrived at the conclusion that 'the injection considerably increases the rate of secretion obtained by a stimulation of given strength of the chorda tympani[2].'

4. SUBLINGUAL SALIVA.

According to Heidenhain[3], the sublingual saliva of the dog forms a viscous mass to which the term fluid can scarcely be applied, and which bears a resemblance to frog's spawn. Sublingual saliva is free from all turbidity, being perfectly transparent and clear. It generally contains a large number of amoeboid corpuscles. The 'paralytic' saliva of the sublingual gland is about as viscous as the normal chorda-saliva, and, therefore, very different in character from paralytic submaxillary saliva.

> "The very viscous condition of sublingual saliva is the result of a kind of 'clotting' of the saliva. When the chorda tympani is stimulated in a dog, the sublingual saliva obtained is usually not especially thick, but in a short time it turns to a jelly, and a little clear watery fluid may be pressed out. When the 'jelly' is obtained from the duct, it is probably because the saliva has clotted there (Langley[4])."

The only analyses of sublingual saliva with which the author is acquainted are four made by Werther and a single one by Langley and Fletcher. Werther, as the result of a research undertaken under the direction of Heidenhain, has drawn attention to the remarkably interesting fact that the extraordinary viscidity of sublingual as contrasted with submaxillary saliva does not depend, as had been surmised, on its containing a much larger proportion of organic solids and especially of mucin, for the amount of water in sublingual saliva fluctuates approximately between the same limits as in the case of the submaxillary secretion, and the proportion of organic constituents in the former is appreciably less than in the latter. The cause of the greater viscidity of the sublingual saliva is believed by Werther to be its neutral or barely alkaline reaction, carbonate of soda not occurring in determinable quantity amongst its mineral constituents. The viscidity of solutions of mucin is known to be connected with, or to depend upon, the alkalies which they contain, for if these be neutralised the solutions become more and more diffluent until the point at which excess of acid precipitates mucin. Whilst the organic matters are in small amount, the proportion of other salts and especially of NaCl in sublingual is much larger than in submaxillary saliva. Whilst the amount of sodium chloride in the blood amounts, according to Sertoli, to 0·59 per cent., in sublingual saliva the chlorine present, calculated as sodium chloride, corresponds to 1 per cent. of this salt.

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[1] J. N. Langley and H. M. Fletcher, 'On the Secretion of Saliva. Chiefly on the secretion of salts in it.' *Philosophical Transactions*, Vol. 180 (1889) B, pp. 109—154. See p. 129.

[2] J. N. Langley and H. M. Fletcher, *Op. cit.* p. 134.

[3] Heidenhain, *Studien des physiol. Instituts zu Breslau.*

[4] Privately communicated to the author.

Appended is a table drawn from Werther's memoir which exhibits the amount of the saliva of the parotid, submaxillary and sublingual saliva collected in stated times, together with the results of their analysis[1].

5. THE SECRETION OF THE GLANDS OF THE MUCOUS MEMBRANE OF THE MOUTH AND TONGUE.

Opening on the surface of the mucous membrane lining the lips and cheeks are certain compound racemose glands, the so-called labial and buccal glands; in addition, large numbers of glands, some of which have the structure of serous, others of mucous glands, open on the surface of the mucous membrane of the posterior part of the tongue. The combined secretion of all these glands adds itself to the fluid poured into the mouth by the salivary glands. We have not, unfortunately, any reliable information upon the character of the mixed secretion, much less as to that of any one of these sets of glands.

The only researches which have been carried out on this subject have consisted in preventing the secretions of the salivary glands from reaching the mouth, either by ligature of their ducts, or by causing them to discharge their contents externally, and examining the secretion then present in the mouth.

Under these circumstances the mouth has been found to be unusually dry, and a small quantity of tough mucus is secreted. Bidder and Schmidt[2] found this to have the following composition:

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[1] Moritz Werther, 'Einige Beobachtungen über die Absonderung der Salze in Speichel' (Aus dem physiologischen Institut zu Breslau). Pflüger's *Archiv*, Vol. 38 (1886), p. 293.

[2] Bidder and Schmidt, quoted by Kühne (*Lehrbuch der Physiologischen Chemie*, p. 16), and by Maly. The author has been unable to discover the original source, as it does not occur in the classical work by these Authors entitled *Die Verdauung nach Versuchen*.

RESULTS OF THE ANALYSES OF PAROTID, SUBMAXILLARY AND SUBLINGUAL SALIVA OF THE DOG. (WERTHER.)

		Time during which secretions were collected.	Amounts collected in grammes.	Water.	Total Solids.	Organic Constituents.	Mineral Constituents.	Salts soluble in H_2O.	Salts insoluble in H_2O.	Alkalinity calculated as Na_2CO_3.	Amount of Cl calculated as NaCl.
Dog 1	Submaxillary Saliva	nearly 2 hours	22·84	98·77	1·23			0·56		0·16	0·335
	Parotid Saliva		22·40	99·14	0·86			0·56		0·19	0·239
	Sublingual Saliva		1·56	97·88	2·12			1·10		0	0·706
Dog 2	Submaxillary Saliva	1 hour	20·38	98·87	1·13	0·66	0·47	0·43	0·042	0·17	0·150
	Parotid Saliva		20·51	99·26	0·74	0·06	0·68	0·64	0·045	0·17	0·078
	Sublingual Saliva		2·05	98·47	1·53	0·19	1·34	1·27	0·068	0	1·080
Dog 3	Submaxillary Saliva	1¾ hours	20·69	98·32	1·68	1·02	0·66	0·58	0·073	0·11	0·329
	Parotid Saliva		16·40	99·26	0·81	0·40	0·41	0·36	0·054	0·17	0·085
	Sublingual Saliva		9·33	98·63	1·37	0·43	0·94	0·90	0·044	0	0·814
Dog 4	Submaxillary Saliva	not stated	40·53	98·74	1·25	0·62	0·64	0·60	0·042	Barely alkaline.	
	Sublingual Saliva		12·63	98·72	1·28	0·34	0·94	0·93	0·017		

(Buccal Mucus).

Water in 1000 parts . . .	990·02
Solids	9·98
Organic matters soluble in alcohol	1·67
„ „ insoluble „	2·18
Salts containing chiefly sodium chloride and phosphate	6·13

According to Jacubowitsch[1] this fluid has no diastatic action.

The glands of the mucous membrane covering the tongue of the frog secrete a viscid liquid endowed, according to Lépine, with diastatic enzyme.

SECT. 3. THE DIASTATIC ENZYME OF SALIVA AND THE ACTION WHICH IT EXERTS UPON STARCH.

Historical Sketch.

Discovery of the amylolytic action of saliva.

It was Leuchs[2] who ascertained that when saliva is mixed with starch it gradually dissolves it with the formation of a body which possesses the reactions of grape-sugar. Schwann[3] confirmed this discovery, the truth of which soon received general assent, though the great majority of scientific men declared themselves of the opinion that in the living organism the saliva could not exert this action to an appreciable extent, and that its function depended essentially upon its watery character aiding gustation, mastication and deglutition[4].

Researches of Mialhe.

In 1845 Mialhe[5] discovered that when filtered human saliva is mixed with five or six times its weight of absolute alcohol, a small quantity of a flocculent body is deposited, which he collected and dried at the temperature of the air. This body he found to be insoluble in strong alcohol, but partly soluble in water and very weak alcohol. He discovered its remarkable property of converting boiled starch into sugar, and from the resemblance to, indeed the apparent identity with, the amylolytic ferment which Payen and Persoz had lately separated from germinating barley[6], he applied to it the name of *animal* or *salivary*

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[1] Virchow in *Ann. d. Charité.* Berlin, 1858.

[2] Leuchs, Kästner's *Archiv,* 1831, quoted by Frerichs in his article 'Verdauung' in Wagner's *Handwörterbuch d. Physiol.*, Vol. III. Part I. p. 768.

[3] Schwann, Poggendorff's *Annalen*, Vol. XXXVIII. p. 358.

[4] Consult Frerichs's brief sketch of the history of this subject (see note 2).

[5] Mialhe, 'De la Digestion et de l'Assimilation des Matières sucrées et amiloïdes.' *Comptes Rendus des séances de l'Académie des Sciences.* Vol. XX. Part I. p. 954. It must be remembered that long before the researches of Payen and Persoz, Kirchhof had observed that in the process of malting, a sugar is produced. Consult Brown and Heron, 'Contributions to the History of Starch and its Transformations.' *Journ. Chem. Soc.*, Vol. XXXV. p. 596 et seq.

[6] Payen et Persoz, 'Mémoire sur la Diastase, et les principaux Produits de ses Réactions.' *Comptes Rendus*, and *Annales de Chimie et de Physique*, Vol. LIII. (1833), p. 73.

diastase. He announced that one part of this body was able to convert 2000 times its weight of starch into sugar.

Early views as to the action of diastatic ferments.

Early in the century (1811) Kirchof discovered that when starch is boiled with dilute sulphuric acid a sugar is formed, and that the same change occurs in the process of malting, by the action, as he thought, of the vegetable albumin upon the starch.

It was soon found that in this process a gum-like body was produced which was subjected to investigation by Biot and Persoz, and, because of its optical properties, denominated by them *dextrin.*

The latter body was discovered to be an isomer of starch, and the sugar formed was supposed to be identical with grape-sugar, which was considered to differ from starch merely by containing one molecule of water in addition. The view which came to be generally entertained was the following: that the first stage in the action either of dilute acids aided by heat, or of diastase, consisted in the transformation of starch into its isomer, dextrin; the second in the transformation of dextrin into grape-sugar.

Researches of Dubrunfaut and O'Sullivan on maltose.

The view that the sugar generated under the action of diastase upon starch was an isomer of, and identical with, grape-sugar was contended against in 1847 by Dubrunfaut[1], who recognised it as a new sugar, to which he gave the name of Maltose. Subsequent elaborate investigations of O'Sullivan[2] established Maltose to be an isomer of cane-sugar, possessing a very different crystalline form, reducing power, and rotatory power to grape-sugar, and still more recent researches have shewn that Maltose is generated not only under the influence of diastase upon the starches but also of the diastatic ferments of the saliva and pancreas[3].

Researches of Musculus.

The assumed identity in the reactions which take place when dilute sulphuric acid and when diastase act on a warm solution of starch was shewn by Musculus to be false.

When diastase acts upon starch the process, according to Musculus, is one not of mere hydration but of decomposition, in which the starch molecule, which he supposes to be of great complexity, splits up into a dextrin and a sugar (which he afterwards admitted to be maltose); a further action causes the dextrin formed to split up again into a less complex dextrin and sugar, the process being repeated until ultimately there result, as products of the reaction, a certain amount of dextrin which has resisted the influence of the diastatic ferment, though it is convertible into sugar by warm dilute acids.

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[1] Dubrunfaut, *Ann. Ch. Phys.* Ser. 3, Vol. XXI. p. 178.

[2] O'Sullivan, *Journal of the Chemical Society*, 2nd Ser., Vol. X. p. 579.

[3] V. Mering and Musculus, 'Ueber die Einwirkung von Speichel- und Pancreasferment auf Glycogen und Stärke.' *Zeitschrift f. physiolog. Chemie*, Vol. I. (1878), p. 395. Also 'Ein Beitrag zur Chemie der Stärke.' *Ibid.*, Vol. II. p. 177.

More recent researches of Brown and Heron.

The researches of Brown in conjunction with Heron[1] and with Morris[2][3] subsequently confirmed and extended those of Musculus and his co-workers, and have thrown additional light upon the complex chemical structure of the starch molecule.

Attempts to separate the Diastatic Enzyme of Saliva.

The methods employed by Mialhe in his attempts to isolate the diastatic principle of the saliva have been referred to (p. 36).

Cohnheim's method.

Subsequently Cohnheim[4] employed the following method:—

The mixed saliva of man is strongly acidulated with phosphoric acid, and lime-water added until the reaction is alkaline and a copious precipitate of Ca_3PO_4 is obtained. This precipitate carries down all the proteids and all the diastatic ferment which the saliva contains. On treating the precipitate with water, it dissolves the ferment which is precipitated by alcohol in the form of white flocculi, which when dried in vacuo yield a nearly colourless powder containing some alkaline phosphates. From the latter it can be purified by repeated solution in water and precipitation with alcohol. The body at last obtained is nitrogenous; it is easily soluble in water, and its solution possesses in a marked manner the diastatic power of the original saliva. The solution is said not to exhibit the xantho-proteic reaction; it is not precipitated by solution of mercuric chloride, platinum tetrachloride, by tannin or by nitric acid, but by neutral and basic lead acetates.

These reactions appear to shew that the diastatic ferment of the saliva, whatever its exact nature, does not possess the properties of a proteid body.

Separation of a diastatic ferment from the tissue of the salivary glands.

In the case of animals whose saliva is endowed with amylolytic properties (e.g. the pig), the enzyme may be extracted from the finely divided salivary glands by digestion in glycerin. From its solution in glycerin the ferment may be precipitated by alcohol, and it may afterwards be dissolved in water.

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[1] Brown and Heron, 'Contributions to the History of Starch and its Transformations.' *Journal of the Chemical Society*, 1879 (*Transactions*), p. 596.

[2] Brown and Morris, 'On the Non-crystallisable products of the action of Diastase upon Starch,' *Journal of the Chemical Society*, 1885 (*Transactions*), p. 527.

[3] Brown and Morris, 'The Determination of the Molecular Weight of the Carbohydrates,' *Journal of the Chemical Society*, 1889 (*Transactions*), p. 462.

[4] Cohnheim, 'Zur Kenntniss der zuckerbildenden Fermente.' Virchow's *Archiv*, Vol. XXVIII. (1865), p. 241.

The pure diastatic ferment yet unknown. There can be no doubt, however, that neither by Mialhe's nor by Cohnheim's method is it possible to obtain the pure salivary ferment. In this case as in that of all other unformed ferments, our methods merely enable the experimenter to obtain substances or extracts which possess in an intense degree the activity of the glands or juices which yield them.

Brief outline of the Changes which Starch undergoes under the influence of the Salivary Ferment.

When saliva acts upon unboiled starch grains, it exerts for a long time no action upon it; in order to insure any conversion of the unboiled starch contact must be prolonged for days. The changes which occur when saliva acts upon boiled starch have, by the researches of Musculus and v. Mehring and of H. T. Brown, been shewn to be similar to, and apparently identical with, those which diastase produces.

Preparation of a suitable solution of starch-paste for experimental purposes. In investigating the action of saliva or any other liquid which contains a diastatic ferment it is convenient to be provided with a well-made starch-paste.

This is best made[1] by using pure potato-starch. The potato-starch is well washed with water, and treated successively with a very dilute solution of potassic hydrate and a 1 per cent. solution of hydrochloric acid; it is then washed with water until the last trace of acid has disappeared and dried at 25°C. A portion of this starch is thoroughly mixed in a mortar with cold water, and the thick liquid then poured with constant and rapid stirring into boiling water and the process of boiling continued for two or three minutes.

The most suitable quantity of starch to be used per 100 c.c. is, according to Brown and Heron, from 3 to 5 grammes, according to Dr Roberts, 1 gramme. The *standard starch mucilage* used in the process of 'diastasimetry' of this author is of the latter strength.

Conversion of Gelatinous into Soluble Starch (Amylodextrin).

The first step in the action of the ferment upon gelatinous starch consists in the conversion of the latter into soluble starch, an action which is accompanied by the liquefaction of the paste.

This liquefaction takes place, at a suitable temperature, with extreme rapidity (almost instantaneously), providing the quantity of ferment be sufficient.

The soluble starch, which is the first product of the action of diastatic ferments, is, like insoluble starch, coloured blue by iodine.

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[1] Brown and Heron. *Op. cit.*, note 1, p. 601.

It is precipitated from its solutions by tannic acid and by alcohol. The property of being precipitated by tannic acid permits of the separation of soluble starch from the dextrins and maltose which are formed at further stages of the reaction.

By adjusting the proportion of diastatic ferment to starch-paste and testing sufficiently soon, it will be discovered that at a certain stage, liquefaction has been the only result, neither dextrins nor sugar being yet present.

Production of Erythrodextrins and Sugar.

When the ferment has acted longer upon starch solution, iodine produces a violet or red colour. At the same time the solution is found to contain dextrins and a sugar. By adding tannic acid to the solution if violet, the yet undecomposed soluble starch is precipitated and then a liquid of a more or less deep red colour is obtained. This liquid contains a body, or bodies, isomeric with starch, precipitable from the solution by the addition of alcohol, and to the product of precipitation the name of erythrodextrin has been given. It has been surmised that more than one erythrodextrin exists. That a definite body erythrodextrin actually exists appears, however, very doubtful, the reactions which were held to prove its existence being explainable on the hypothesis that it is a mixture of varying quantities of unaltered starch with achroodextrin and maltose.

Erythrodextrin, or the mixed product so named, is soluble in water, precipitable from its solution by alcohol, but not by tannic acid.

The solution is coloured red by iodine.

When erythrodextrin is subjected to the further action of a diastatic ferment it is decomposed into two isomeric dextrins of different reactions, termed achroodextrin and maltodextrin, and into maltose.

Production of Achroodextrin, Maltodextrin and Maltose.

When a starch solution is subjected at a suitable temperature to the prolonged action of a diastatic ferment, the clear solution is found not to be coloured red by iodine, but to assume a yellow tinge, which becomes gradually fainter until no colouration is produced by the reagent. This is the so-called '*achromic* point' of Dr Roberts. By causing a large quantity of diastatic enzyme to act upon a sufficiently dilute starch mucilage, the whole of the stages of the transformation, culminating in the *achromic* point, can be brought about almost instantaneously.

Influence of temperature upon diastatic action of saliva.

Dr Roberts's experiments have led him to the conclusion that the diastatic activity of saliva increases with rise of temperature up to about 30° C., and that it continues steady from this temperature to about 45° C. and then declines, being finally extinguished between

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65° and 70°. The salivary enzyme would appear to be influenced by temperature exactly as the diastatic enzyme of the pancreas.

Influence of the quantity of enzyme present on the amount of starch converted.

In the case of the diastatic enzyme of the pancreas Roberts has shewn that the amylolytic work done by a given solution containing it is strictly proportional to the quantity of it set in action; in other words, the amount of the standard starch mucilage which can be changed to the '*achromic point*' in a given time and at a given temperature, varies directly as the quantity of the solution employed. This law appears to hold equally well in the case of the salivary enzyme.

Influence of the quantity of enzyme on the time occupied in the transformation.

Within certain limits the time occupied in effecting the transformation varies inversely as the quantity of the enzyme or enzymic solution, i.e. double the quantity of an enzyme; and the transformations will occur in half the time.

The evidence of the existence of various products.

When alcohol is added to a starch solution subjected to the action of ptyalin, or to that of any other diastatic ferment, at any stage of the process, there is thrown down a precipitate which is composed of a mixture of dextrins, whilst the filtrate contains maltose. At different stages of the ferment-process, the solution exhibits changes in its power of reducing cupric oxide (as determined by boiling a given volume of it with Fehling's solution), and in its power of rotating the plane of polarised light, the former increasing and the latter diminishing as the process proceeds; similarly the products which can be precipitated at various stages differ in their reducing and rotatory powers.

In investigating the soluble products of the action of a diastatic enzyme on a starch solution, at any particular stage of the process, a known volume of the filtered solution is concentrated by evaporation in the water bath, and absolute alcohol is then added in such proportion as to furnish a mixture containing about 95 per cent. of absolute alcohol. By this procedure the dextrins present are entirely precipitated, and their amount determined by collecting them on a weighed filter, washing with absolute alcohol, drying at 100° C. and weighing. The alcoholic filtrate, which contains the whole of the maltose, is evaporated to dryness, the residue dissolved in distilled water, and the amount of sugar determined (*a*) by Fehling's solution, preferably by weighing the oxide of copper formed: (*b*) by means of the Polarimeter. The following is an example (taken from a memoir by Dr Lea[1]) which illustrates the accuracy with which by careful experimenting, the amounts of the products can be determined.

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[1] Sheridan Lea, 'A Comparative Study of Artificial and Natural Digestion. *Journal of Physiology*, Vol. XI. 1890, p. 234.

3·412 grammes of starch boiled in 100 c.c. of water were digested at 40° C. with 100 c.c. of dilute human saliva for 15 hours, and their products subjected to the previously described process:

3·412 grammes starch yielded	0·505 grammes	dextrin
	2·838 „	maltose
	3·343	

Whilst the nature and characters of the sugar which is produced are not the subject of dispute, considerable divergence of opinion exists as to the number and the character of the individual dextrins which are the ultimate products of the action of a diastatic ferment on starch. The most recent researches on the subject, by Brown and Morris, have led these observers to the conclusion that the physical and chemical properties of the different dextrins precipitable at several successive stages of the process may be accounted for on the supposition of their being mixtures of maltose with one definite achroodextrin which is entirely free from reducing power and which has a specific rotation $(\alpha) D = 194°\cdot8$ and $(\alpha) j = 216°$. The above dextrin was obtained in a state of freedom from maltose by subjecting it to the action of Knapp's reagent, viz. heating it with a solution of cyanide of silver in solution of sodium hydrate.

Maltodextrin. In addition to this achroodextrin and maltose, and having properties which more nearly resemble the latter than the former, there occurs, at least under certain circumstances, a body first obtained in an impure condition (i.e. contaminated with maltose) by Herzfeld, which is possessed of reducing powers, whose specific rotation is for $(\alpha) j = 193°\cdot1$ and for $(\alpha) D = 174°\cdot5$; to this body Brown and Morris retain the name of maltodextrin ascribed to it by Herfeld.

Is Maltose the only Sugar produced by the action of Saliva on Starch? This question cannot be answered with complete precision. Brown and Heron[1] have shewn that the diastatic ferment of pancreatic juice possesses the property, *which is not possessed by malt-diastase*, of transforming a small proportion of maltose into dextrose, when the action is prolonged. Reasoning on the ground of the apparent identity of the actions of the Salivary and Pancreatic diastatic ferments in their mode of action on starch, we should be inclined to surmise that the ultimate products resulting from the two ferments would be the same. It must be stated, however, that in his research Lea did not obtain evidence of the formation of any sugar but maltose.

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[1] Brown and Heron, 'Some Observations upon the Hydrolytic Ferments of the Pancreas and small Intestine.' *Proceedings of the Royal Society*, 1880, p. 393.

The following list exhibits the products of the action of diastatic ferments on starch, with certain of their distinguishing characters.

SPECIFIC ROTATION, REDUCING POWER AND REACTION WITH IODINE OF THE CHIEF PRODUCTS OF THE ACTION OF SALIVA ON STARCH.

	Specific Rotation[1].	Reducing Power.	Reaction with Iodine.
1. Starch in a gelatinous condition (starch-paste)	$\alpha (D)$ 197° $\alpha (j)$ 219·5	Reduces.	Coloured blue by iodine.
2. Soluble starch (amylodextrin) $(C_{12}H_{20}O_{10})_n$	$\alpha (D)$ 194°·8 $\alpha (j)$ 216°·0	Reduces	Coloured blue by iodine.
3. Erythrodextrin $(C_{12}H_{20}O_{10})_n$	? ? ?	Reduces	Coloured of a reddish-violet by iodine.
4. Achroodextrin $(C_{12}H_{20}O_{10})_n$	$(\alpha) D = 194°·8$ $(\alpha) j = 216°$	No reducing power	Not coloured by iodine.
5. Maltodextrin $(C_{12}H_{20}O_{10})_n$	$(\alpha) D = 174·5$ $(\alpha) j = 193·1$	Reduces	Not coloured by iodine.
6. Maltose $C_{12}H_{22}O_{11} + H_2O$ crystalline; largely soluble in water; soluble in alcohol	$(\alpha) D = 137°·0$ $(\alpha) j = 150°·0$	Reduces Fehling's solution (does not reduce a solution of copper acetate in acetic acid—Barfoed's reagent). Cupric oxide reducing powers compared with that of dextrose at 100, is 65.	Not coloured by iodine.

[1] By α (D) is designated the Specific Rotation for light having a wave length corresponding to Frauenhofer's line D. By α (j) we designate the rotation for mean yellow light.

Determination of the cupric oxide reducing power of soluble carbohydrates[1].

"Dextrose being the substance of which the reducing power was first determined, it may be taken as the standard to which to refer all other carbohydrates, the cupric oxide reducing power being the amount of cupric oxide calculated for dextrose $C_6H_{12}O_6$, which 100 parts reduce. Thus the cupric oxide reducing power of dextrose being 100, that of maltose is 65, and that of milk sugar 70.

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[1] This paragraph is taken verbatim from Miller's *Elements of Chemistry*, edited by Professor Armstrong and Mr Groves. 5th Ed. (1880) Vol. III. p. 568.

The cupric solution employed, which is usually termed Fehling's solution, is prepared by dissolving 34·639 grammes of pure crystallised cupric sulphate in about 200 c.c. of water, and in another vessel 173 grammes of pure potassic sodic tartrate—so-called Rochelle salts—in 480 c.c. of a solution of pure sodic hydrate of specific gravity 1·14; the first solution is then added gradually to the second, and the deep-blue coloured clear fluid is diluted to 1000 c.c. It must be kept in a cool, *dark* place, in well-closed bottles, filled to the top, as the action of light or the absorption of carbonic anhydride would lead to the separation of cuprous oxide on mere exposure to heat. Before using the solution, mix 10 c.c. of it with 40 c.c. of water, and boil the mixture for some minutes; if this produces the least change, and causes the separation of even the smallest quantity of cuprous oxide, the solution is unfit for use. Of this solution 25—30 c.c. are poured into a beaker of 130—140 c.c. capacity, together with about 50 c.c. of boiling well-boiled water; the beaker is then placed in a water bath, which is kept boiling, and at the end of five or six minutes, when the dilute copper solution has acquired as nearly as possible the temperature of the bath, a known quantity of the solution to be tested is added, and the heating continued for twelve or fourteen minutes. If the blue colour completely disappears in the first three or four minutes, it can be restored by adding quickly more copper solution, but if two or three additions be necessary to insure an excess, the experiment must be sacrificed, and a fresh one made with a smaller quantity of the carbohydrate solution. Satisfactory results cannot be obtained unless this precaution be adopted: the numbers generally falling too low with solutions of maltose or the glucoses, and too high when dextrin is also present, if the amount of cupric solution employed be not from the first in excess. After thirteen or fourteen minutes heating, the precipitated cuprous oxide is rapidly filtered out, washed with boiling, well-boiled water, dried, and ignited in the usual way; strong ignition in an open porcelain crucible for five or six minutes completely converts the cuprous into cupric oxide, and treatment with nitric acid is unnecessary.

The time of heating above mentioned gives the true reduction for dextrose and maltose, and the quantity of cuprous oxide precipitated remains constant, even if the heating be continued for twenty minutes; but if the solution in addition contains dextrin, the reduction becomes greater, owing doubtless to the slow conversion of the dextrin into substances capable of acting upon the cupric solution (O'Sullivan, *Journ. Chem. Soc.* 1876, II. 130).

The cupric oxide reducing power is often determined volumetrically, with the previously mentioned Fehling's solution, of which 10 c.c. correspond to 0·05 grammes of dextrose. 10 c.c. of the cupric solution and 40 c.c. of water are heated in a water bath kept boiling, and the highly dilute solution of the carbohydrate is added in small quantities from a burette, until the blueish-green colour of the solution entirely disappears. Although concordant results may, with great care, be obtained by this process, the polarimetrical method is far more reliable, and involves very little more trouble in its execution."

The products of ferment-

It is true of all enzymes that the substances which result from their action as they accumulate in the

action act prejudicially on its progress and tend to arrest it.

medium in the act of fermentation, gradually slow, and ultimately arrest the specific process, which may be restored to its original intensity more or less completely by dilution, or still better by separating the soluble ferment products by diffusion. In order to accomplish the latter object, the process of digestion has been carried out in specially constructed dialysers, such as those which we owe to Kronecker (Fig. 2), and that of Sheridan Lea, shewn in the accompanying figures (Fig. 3). In Lea's apparatus the digestive process is

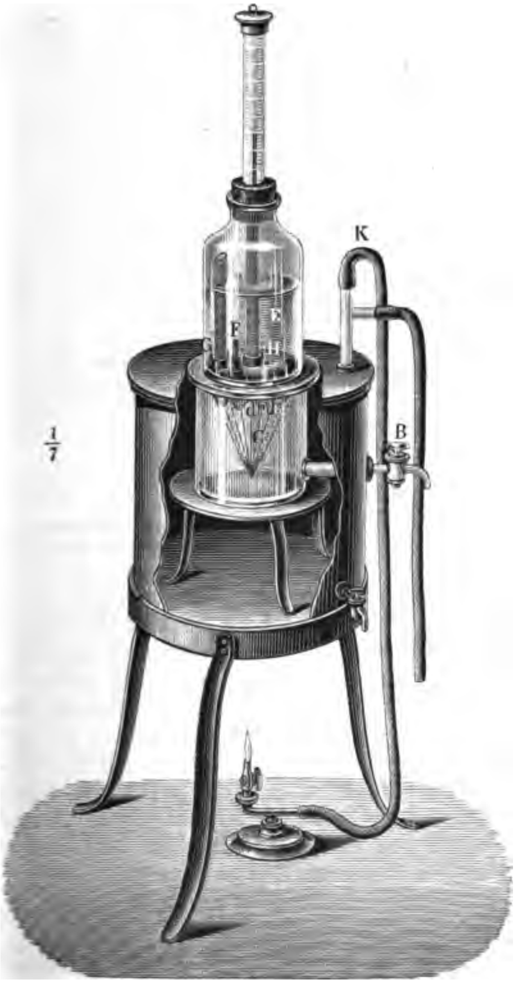

FIG. 2. KRONECKER'S APPARATUS FOR THE SEPARATION BY DIALYSIS OF THE PRODUCTS OF DIGESTION.

carried on in a tube of parchment paper, which is surrounded by liquid which is kept at a constant temperature, the tube being also subjected to a continuously recurring movement, so as to imitate as nearly as possible the conditions which exist in the alimentary canal.

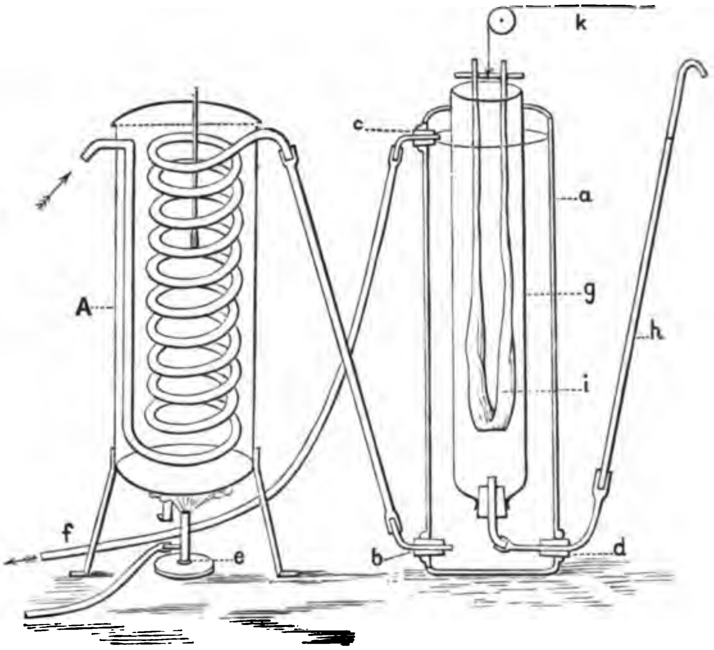

FIG. 8. DR SHERIDAN LEA'S APPARATUS FOR SEPARATING BY MEANS OF DIALYSIS, THE PRODUCTS OF DIGESTION, AND FOR KEEPING THE DIGESTIVE MIXTURE IN MOTION.

"By means of this apparatus a distant and incomplete approximation to two of the more important conditions under which normal digestion takes place may be realised, viz., continuous movement and removal of digestive products. The substance to be digested is placed inside the dialyser-tube (*i*) together with the digestive fluid; (*g*) is filled up to the level of the tubulure (*c*) with a fluid similar in composition to that which is in (*i*) but minus any ferment, and the contents of (*g*) and (*i*) are maintained at any desired digestive temperature by means of the current of water which flows through (*a*). Finally, the dialyser-tube is kept in constant motion by the string (*k*)."

"The mixing of the contents of (*i*) is very perfect, waves which might almost be called 'peristaltic' running up the flexible walls of the dialyser-tube each time it is suddenly lowered after its more gradual ascent. The removal of the digestive products is on the one hand incomplete and falls far short of the activity of the removal existing in normal digestion, for it is dependent merely upon the diffusibility of the products. In the body, on the other hand, we have now every reason for regarding the absorption of products as dependent primarily upon a specific selective activity of the epithelium lining the alimentary canal, and not merely upon the physical properties of the substances to be absorbed. So far, however, as diffusion is the only obvious means at our disposal for removing the digestive products, the dialyser above described is extremely efficient[1]."

The Sugar which results from the action of Saliva on Starch, MALTOSE.

However large the quantity of diastatic enzyme present in a starch solution and however favourable the circumstances, under ordinary circumstances, *in vitro*, the conversion into maltose does not proceed beyond a certain point. The tendency of all such transformations is to proceed at first speedily and to attain a point of equilibrium beyond which further progress is relatively very slow. This point of equilibrium in the case of starch acted upon by malt extract *under the conditions* in which Brown and Heron experimented was reached when the mixed products possessed a specific rotation $(\alpha)j = 162{\cdot}6$, and a reducing power $\kappa = 49{\cdot}3$, properties which correspond to a mixture composed of

Maltose 80·9
Dextrin 19·1,

and which *might* be expressed by the reaction

$$10\,\underbrace{C_{12}H_{20}O_{10}}_{\text{Starch}} + 8\,H_2O = 8\,\underbrace{C_{12}H_{22}O_{11}}_{\text{Maltose}} + 2\,\underbrace{C_{12}H_{20}O_{10}}_{\text{Dextrin}}$$

Lea has however shewn that if conditions resembling those which presumedly exist in the alimentary canal be realized, viz. if the solution of starch be not too concentrated, if the diastatic ferment be active and the sugar formed removed, there is no such limit to the transformation of starch as indicated by the above equation so that conversion into sugar tends to be complete.

Properties of maltose.

Maltose, for which the terms *amyline* or *starch-sugar* have also been proposed, crystallises from water or alcohol in white crusts composed of fine needles. It is very soluble in water, but much less so in alcohol. It is isomeric with cane sugar, but its crystals possess one atom of water of crystallisation ($C_{12}H_{22}O_{11} + H_2O$), which is expelled at 100° C.

Maltose is dextro-rotatory and like glucose reduces Fehling's solution.

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[1] Sheridan Lea, 'A Comparative Study of Artificial and Natural Digestion.' *Journal of Physiology*, Vol. XI. (1890, p. 227 et seq.).

Its higher optical activity and smaller reducing power as compared with glucose is exhibited below.

	Reducing Power.	Optical Activity. $(\alpha)j$
Glucose	100	53[1]
Maltose	65	+ 150°

Maltose is easily and completely fermented by yeast. When a mixture of maltose and sucro-dextrose or glucose is fermented, the whole of the former is said to disappear before the latter is touched[2]. When heated for 3 hours with very dilute sulphuric acid, maltose yields 98·3—98·9 of its weight of dextrose.

By the action of dilute sulphuric acid upon maltose at temperatures between 80° and 90° C., there is obtained, as the only product, a dextrose having the specific rotation $(\alpha)j = 58^\circ{\cdot}65$, and a cupric reducing power of 100[3].

When boiled with a solution of potassium or sodium hydrate, solutions of maltose react as solutions of dextrose, assuming a yellow amber colour which gradually deepens into a dark yellowish brown as the process of boiling is continued.

Similarly, maltose resembles dextrose in its behaviour when boiled with a solution of sodium carbonate and basic bismuth nitrate, or bismuth carbonate; the bismuth is reduced and the powder suspended in the liquid becomes brown.

With Phenyl-hydrazin $NH_2 - NH\,(C_6H_5)$ maltose, like the other true sugars, forms a compound; a so-called phenyl-maltosazon, which separates in clusters of yellow crystals; the compound which consists of two molecules of maltose and two molecules of phenyl-hydrazin, has the composition $C_{24}H_{32}N_4O_9$: is soluble in about 75 parts of hot water and melts at about 206°[4]. Whilst in its melting point this compound resembles the analogous glucosazon, $C_{18}H_{22}N_4O_4$ (which melts at 205° C.), which is formed by the action of phenyl-hydrazin on grape sugar, it is distinguished from it by the fact that the latter compound is almost insoluble in water.

In order to obtain this interesting compound of Phenyl-hydrazin and maltose the general process employed in the preparation of all the osazones is followed. Dissolve 2 grammes of phenyl-hydrazin hydrochloride with twice its weight of sodium acetate in 20 c.c. of distilled water.

To a fairly concentrated solution of maltose add an equal volume of this solution and place the mixture in a water bath, keeping it at 100° C. for half-an-hour. The liquid assumes a yellow colour and if sufficiently concentrated commences to deposit the crystalline maltosazone; on allowing it to cool the deposit increases in amount. Under the microscope clusters of yellow crystals are observed.

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[1] The rotatory power of glucose solutions varies with their concentration and temperature, diminishing as the concentration is less and vice versa (Tollens).

[2] Miller's *Chemistry*, Vol. III. p. 618.

[3] Brown and Heron, *Op. cit.*, p. 620.

[4] Emil Fischer, 'Synthesen in der Zuckergruppe.' *Berichte d. deutsch. chem. Gesellschaft.* Berlin, 1890 (No. 12), S. 2119.

When boiled for a short time with a solution of cupric acetate containing free acetic acid, maltose does not reduce it, whilst glucose under the same circumstances partially reduces the solution.

Barfoed's Reagent, which is employed as a distinguishing test between maltose and dextrose, is a solution of 1 part of cupric acetate in 15 parts of water; to 200 c.c. of this solution 5 c.c. acetic acid, of 38 per cent., are added.

Theoretical views as to the action of diastatic ferments on starch.

From the facts which have been enunciated it has been surmised by Musculus and Gruber[1], and by Brown and Heron, that starch is a polysaccharide having a formula $(C_{12}H_{10}O_{10})_n$, which under the influence of hydrolytic agencies undergoes successive hydrations and decompositions.

As a result of their researches, Brown and Heron described a series of erythrodextrins and achroodextrins, of gradually diminishing molecular weight, which they believed to be formed during the process of diastatic hydrolysis, each complex dextrin splitting up into a molecule of maltose and a molecule of a dextrin of smaller molecular weight, and of less complexity. The more recent researches of Brown in conjunction with Morris have not however confirmed this hypothesis. According to these researches all the intermediate products which may be separated from a solution of starch which is being acted upon by a diastatic ferment, may be accounted for as compounds of a non-reducing dextrin with maltose. At the same time, as has been already mentioned, there appears under certain circumstances to be formed a body termed malto-dextrin which stands in near relation to the non-reducing dextrin and to maltose, and which, under the influence of a diastatic enzyme, readily yields maltose.

Brown and Morris believe that the molecule of starch cannot consist of less than an aggregation of 5 times the molecule $(C_{12}H_{20}O_{10})_3$. They would represent the starch molecule as

$$\left\{\begin{array}{l}(C_{12}H_{20}O_{10})_3\\(C_{12}H_{20}O_{10})_3\\(C_{12}H_{20}O_{10})_3\\(C_{12}H_{20}O_{10})_3\\(C_{12}H_{20}O_{10})_3\end{array}\right.$$

Under the action of a diastatic ferment the complex molecule gradually is degraded, by successive removals of the ternary groups.

The removal of each $(C_{12}H_{20}O_{10})_3$ group is accomplished prior to its complete hydration, the ternary compound being split off in the form of malto-dextrin, when one of its sub-groups has been hydrated, thus:—

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[1] Musculus u. Gruber, 'Ein Beitrag zur Chemie der Stärke.' *Zeitschr. f. physiol. Chem.*, Vol. II. pp. 177—190.

$$\underbrace{(C_{12}H_{20}O_{10})_3}_{\text{One of the five ternary groups constituting the hypothetical starch molecule.}} + H_2O = \underset{\text{Malto-dextrin.}}{\left\{\begin{matrix} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_2 \end{matrix}\right.}$$

Under the influence of fresh diastatic ferment, acting under favourable conditions, malto-dextrin readily undergoes the change indicated in the following equation:

$$\underset{\text{Malto-dextrin.}}{\left\{\begin{matrix} C_{12}H_{22}O_{11} \\ (C_{12}H_{20}O_{10})_2 \end{matrix}\right.} + 2H_2O = \underset{\text{Maltose.}}{3C_{12}H_{22}O_{11}}.$$

These hypotheses, advanced to explain the action of malt extract on starch, will apply *mutatis mutandis* to the changes induced by any diastatic enzyme.

Influence of certain poisonous agents on diastatic action.

Carbolic acid, unless when present in the proportion of 5 per cent. of the starch jelly, does not interfere with the diastatic action of saliva.

Salicylic acid possesses, on the contrary, a much more powerful action. According to Julius Müller[1], salicylic acid in the proportion of 0·2 per cent., retards the action of saliva upon starch-paste, whilst in the proportion of 1 per cent. it arrests it. Although, therefore, susceptible in a high degree to the action of salicylic acid, the diastatic salivary ferment is not so much so as diastase, as will be again referred to in the sequel.

Some poisons, as arsenious acid[2], possess no power of influencing the diastatic ferment of saliva; others, such as hydrocyanic acid, possess this power only in a feeble degree and when present in large quantities.

Is the diastatic ferment of the saliva identical with malt-diastase?

Although the ultimate products of the action of the diastatic ferment of saliva and vegetable diastase appear to be identical, there are facts which conclusively prove the individuality of these two bodies.

The following are certain points of difference between malt-diastase and salivary ferment.

1. Malt-diastase acts powerfully upon starch through a wide range of temperatures; its activity is greatest at 60° C., and then

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[1] Müller, 'Ueber die antiseptischen Eigenschaften der Salicylsäure, gegenüber der Carbolsäure.' *Journ. f. prakt. Chemie*, New ser., Vol. x. p. 45.

[2] Schäfer and Böhm, 'Ueber den Einfluss des Arsens auf die Wirkung der ungeformten Fermente.' *Verhandl. d. physik.-med. Gesellsch. in Würzburg*, N. F. Vol. III. p. 238.

commences to decline, not being entirely destroyed till the temperature approaches 80° C.

It has been shewn, however, by O'Sullivan and by Brown and Heron that the changes which are brought about by malt-diastase are influenced in a very marked manner by temperature.

The range of temperature at which the salivary ferment acts most powerfully is according to Roberts very wide, viz. from 30°—45°. According to Kjeldahl the most favourable temperature is 46° C.[2] The ferment is however destroyed when its solutions are heated to between 65° and 70° (Roberts), i.e. at a temperature 10 degrees lower than that which destroys the action of malt.

2. Salicylic acid when present in the proportion of 0·05 per cent. at once stops all action of malt-diastase upon starch-paste. In such proportions, it exerts no perceptible action on the salivary ferment. It is only when present in the proportion of 0·1 per cent. that the *least* slowing influence is perceptible, and as much as 1 per cent. must be present in order to arrest entirely the action of the ferment.

SECT. 4. EXCRETION OF MEDICINAL SUBSTANCES IN THE SALIVA.

Certain medicinal agents, as potassium iodide, are excreted in the saliva. Others are not, e.g. potassium ferrocyanide. Mercury, of which medicinal preparations induce, under certain circumstances, profuse salivation, has been detected in saliva.

It is said that lead can be detected[3] in the saliva of persons suffering from lead-poisoning, in whom salivation has been induced by injection of pilocarpin. Under similar circumstances arsenic has not been detected.

Rapidity of secretion of certain salts when injected into the blood.

Langley and Fletcher found (1) on injecting 50 c.c. of a solution of lithium nitrate into the blood that the first drop of saliva secreted both from the submaxillary and from the parotid gland shewed the lithium band in the spectroscope; (2) on injecting 50 c.c. of a solution of potassium iodide into the blood, the salt was present in all the drops of saliva after the first six, appearing first in the submaxillary secretion, which flows more rapidly than the parotid, although the quantity of iodide was larger in the parotid than in the submaxillary saliva.

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[1] Kühne, *Lehrbuch*, p. 21.

[2] Kjeldahl, 'Untersuchungen über zuckerbildende Fermente.' Abstracted from the original Swedish by Hammarsten, Maly's *Jahresbericht*, Vol. IX. p. 381.

[3] Pouchet, 'Recherche des substances médicamenteuses et toxiques dans la Salive.' *Comptes Rendus*, Vol. LXXXIX. p. 244.

[4] Langley and Fletcher, *Op. cit.* pp. 149 and 150.

SECT. 5. CHANGES WHICH THE SALIVA UNDERGOES IN DISEASE. SALIVARY CONCRETIONS.

From the difficulties and inconvenience which attend the collection of large quantities of saliva but little knowledge is possessed of the changes which it undergoes in disease. Our information is limited almost entirely to that which relates to the passage of abnormal ingredients into the secretion.

Saliva in diabetes.

In diabetes, the saliva, which was formerly supposed to contain sugar, is said to be free from sugar, but to contain lactic acid, and to have, in consequence, an acid reaction.

The Author has examined the saliva collected in two cases of diabetes, after the subcutaneous injection of pilocarpin. The first was that of a diabetic passing large quantities of highly saccharine urine, and charged with the acetone-like body which gives a red colour with ferric chloride; this patient succumbed to an attack of diabetic coma a few days after the examination of the saliva. The saliva had *a very marked alkaline reaction.* It contained no trace of sugar. It contained a trace of sulphocyanates.

In a second case of diabetes, in a woman, the saliva had likewise a very marked alkaline reaction. It contained no trace of sugar.

These two observations are in contradiction to the usual statement that the saliva of diabetics is acid.

Saliva in jaundice.

The colouring matters of the bile and the bile salts are generally stated not to occur[1] in the saliva of persons with jaundice. According to Fenwick, however, their presence may be detected in certain cases[2].

Saliva in Bright's disease.

In diseases of the kidney the amount of albumin in the saliva may be much increased, as has been noticed by Vulpian[3], who examined the saliva of such patients after administering pilocarpin. When in such cases the excretion of urea by the kidneys diminishes, this constituent is found in saliva in much larger quantities than normal[4].

Salivary Concretions.

Salivary concretions occur in the acini and in the ducts of the salivary glands, as well as in the buccal glands. Often they are of small, indeed of microscopical dimensions; sometimes, and then they usually occupy the ducts of the salivary glands, they are much larger, being of the size of a pea and sometimes very much larger.

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[1] See v. Jaksch, *Clinical Diagnosis.* Translated by Dr James Cagney, London 1890, see p. 57.

[2] Fenwick, 'Lecture on the presence of bile in the Saliva.' *Lancet*, 1877, Vol. II. 303.

Fenwick, *The Saliva as a Test for Functional Disorders of the Liver.* London 1887, see p. 11.

[3] Vulpian, 'Augmentation des matières albuminoïdes dans la salive des albuminuriques.' *Comptes Rendus*, Vol. LXXXVIII. p. 1165.

[4] Ritter, abstracted in Maly's *Jahresbericht*, Vol. I. p. 166.

The larger salivary concretions are round or oval, smooth or rough, sometimes of a white, and sometimes of a yellowish grey colour, usually homogeneous, and pulverisable. Sometimes they are hard and stratified, rarely they exhibit a radiated structure and possess a visible nucleus. Rarely, when powdered, they exhibit crystalline fragments. When treated with dilute mineral acids, the mineral salts of the concretion are dissolved, leaving an organic mass. Such concretions usually occur singly in the duct of one of the glands, though more rarely several (as many as ten) are found. They weigh as a rule from 1·5 to 2 grammes, exceptionally as much as 3 or 4 grammes, and occur most frequently in Wharton's duct; in the parotid they occur about ten times less frequently than in the submaxillary gland, and in the sublingual gland they are still rarer[1].' According to Kühne, salivary concretions usually contain the salivary diastatic ferment, so that when powdered they act energetically upon starch.

The following tabular statement[2] exhibits the results of analyses of salivary concretions by various observers:

Constituents in 100 parts.	Wright.			v. Bibra.	Lecanu.	Besson.	Golding-Bird.
	(1)	(2)	(3)				
Calcium carbonate	81·3	79·4	80·7	13·9	20	15	2
„ phosphate	4·1	5·0	4·2	38·2	75	55	75
Magnesium phosphate	—	—	—	5·1		1	—
Soluble salts	6·2	4·8	5·1	38·1			—
Organic matters	7·1	8·5	8·3		5·0	25	23
Water and loss	1·3	2·3	1·7	6·3		—	

Tartar of the Teeth.

The tartar of the teeth is in great part composed of salts which have been deposited from the saliva, and it therefore has much resemblance to salivary concretions. The tartar occurs usually in masses which are of a yellowish, greenish or brown colour. Besides salts they contain mucus, squamous epithelium cells, and long filaments of *Leptothrix buccalis*, all of which are rendered evident if a little of the powdered concretion be treated with dilute hydrochloric acid and the residue be examined microscopically.

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[1] Hofmann, *Lehrbuch der Zoochemie*, Wien, 1879, p. 146.

[2] Gorup-Besanez, *Lehrbuch der physiologischen Chemie*, 4th Ed. (1878), p. 476.

It is said that the composition of the tartar of the incisor teeth differs from that of the molar teeth, the latter containing much larger quantities of phosphate of iron and more silica.

The tartar is as stated above in great part produced by the precipitation of the salts of the saliva; in part however it is produced, doubtless, by the precipitation of lime and iron salts of the food through the agency of the alkaline phosphates contained in the saliva.

The following are analyses exhibiting the composition of Tartar (Vergnes[1]).

	Tartar of the Incisor Teeth.		Tartar of Molar Teeth.	
Phosphate of calcium	63·88	62·56	55·11	63·12
Carbonate "	8·48	8·12	7·36	8·01
Phosphate of iron	2·72	0·82	12·74	4·01
Silica	0·21	0·21	0·37	0·38
Alkaline salts	"	0·14	"	0·31
Organic matter	24·69	27·98	24·40	24·01

SECT. 6. DIRECTIONS FOR THE QUANTITATIVE ANALYSIS OF SALIVA.

Collection. When it is thought desirable to examine saliva of man, care should be taken to collect it an hour or two after a meal, and the precaution should be taken to have the mouth thoroughly rinsed out with water.

Since the discovery of the powerful sialagogue action of jaborandi and its active principle pilocarpin, a subcutaneous injection of the latter would usually be employed in obtaining saliva from the human subject for analysis. Within ten minutes after such an injection salivation occurs so as to admit of considerable quantities being collected.

Determination of reaction. Proceed as directed in Vol. I. p. 26.

Determination of specific gravity. This should be effected by means of the bottle (see Vol. I. p. 174).

Determination of water, total solids and salts. Proceed exactly as indicated in the case of blood; using, however, 20 c.c. of saliva.

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[1] Vergnes, *Du tartre dentaire*, Paris, 1869, 8vo., quoted by Gautier, *Chimie physiologique*, Vol. II. p. 281.

Determination of mucin (with epithelium).

A known weight (30—50 grms.) of filtered saliva is treated with a few drops of acetic acid. Mucin is precipitated; it may be collected on a weighed filter, washed with absolute alcohol and then with ether, and the combined weight of filter and mucin determined.

Determination of Proteids.

All saliva contains, as has been said, some proteid bodies in solution. As the quantity is small and there is always some, usually a preponderating quantity of mucin, the determination does not admit of great accuracy. The following plan is recommended, but is only applicable when considerable quantities of saliva are available.

To 50, or better still 100 grms. of saliva add a few drops of acetic acid; collect the precipitated mucin, following the directions given in the last paragraph. To the filtrate add at least three times its volume of absolute alcohol, and set aside the mixture for 24 hours. Collect the precipitate on a weighed-filter, wash with absolute alcohol, and ether, and then with boiling water. The filter with its contents is then dried, first in the water-oven and then in the air-oven at 120°C., and weighed between watch-glasses. Thus we determine the combined weight of the proteids and of certain of the salts present in the portion of saliva analysed. The filter and contents are then ignited in a weighed platinum capsule, and after the operation the weight of the ash left is determined. On subtracting from the weight of the proteids and ash, as found by the previous operation, the weight of the ash, we obtain the weight of the proteids present.

Determination of the presence and amount of sulphocyanic acid in saliva:—Munk's method.

The presence of sulphocyanic acid is established by the tests referred to at page 19.

The amount of sulphocyanic acid is best determined by the following method, which was carried out by Munk at the suggestion of Salkowski[1]:—

A known weight of saliva is evaporated to dryness, and an alcoholic solution of the dry residue is made; this is evaporated to dryness, dissolved in water, and the clear solution is precipitated by means of silver nitrate and nitric acid; the precipitate is washed, collected on a filter, and dried at 100° C.; it is then ignited in a silver dish, with pure sodium hydrate and potassium nitrate. The fused mass is dissolved in water and the solution is treated with hydrochloric acid and barium chloride, which precipitates the sulphuric acid formed by the oxidation of the sulphocyanic acid. From the weight of the barium sulphate the amount of sulphur originally present in the sulphocyanate is ascertained.

Colorimetrical methods.

The estimation of the amount of sulphocyanic acid in the saliva has been usually carried out by more or less accurate colorimetric methods, i.e., the amount has been deduced by comparing the intensity of the red colouration produced by adding ferric chloride to a known volume or weight of saliva, with the colour produced under exactly similar conditions when the same iron solution is added to solutions containing known quantities of sulphocyanic acid. By employing the method suggested by Hoppe-Seyler for the determination of Hæmoglobin, see Vol. I. p. 182, close approximations may be doubtless arrived at. With the help of one of the recently perfected colorimeters, as that of Dubosque or of C. H. Wolff, still better results would be obtained[1].

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[1] Munk, 'Schwefelcyanbestimmung im Speichel.' Virchow's *Archiv*, Vol. LXIX. p. 350.

Spectro-photometrical method.

Professor Vierordt, employing the method of spectro-photometry to the estimation of sulphocyanates, determined the extinction-coefficient of sulphocyanide of iron, and on the assumption of its constancy and therefore of the possibility of applying spectro-photometry to the solution of the problem, made determinations of the amount of sulphocyanate present in the saliva. Unfortunately it has been shewn by the brothers Krüss that spectro-photometry[2] is not applicable to the determination of sulphocyanate of iron, inasmuch as the nature of the compound formed varies within wide limits, and with these variations likewise varies the extinction-coefficient and, necessarily also, the 'absorption-relation.'

Determination of the Diastatic Value of Saliva. 'Diastasimetry.'

It has been said that the enzyme which confers upon the saliva of man and of some other animals its diastatic powers has not been separated, so that we merely reason concerning the existence of the body from a knowledge of its powers. Whilst we cannot estimate the weight of the unknown body, we may determine the enzymic power of any liquid containing it, the latter doubtless being, *cæteris paribus*, in direct relation to the richness or poverty of the liquid in the body.

Principles of Robert's method of diastasimetry.

The diastatic value of a liquid, represented by the symbol D, may be expressed by the volume in cubic centimetres of a standard solution of starch mucilage which can be transformed by one cubic centimetre of the liquid, acting during five minutes at a temperature of 40° C., to the so-called *achromic* point, the latter being recognized by the non-production of any colour reaction on the addition of iodine.

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[1] For a description of this colorimeter consult 'Kolorimetrie und quantitative Spektralanalyse in ihrer Anwendung in der Chemie,' von Dr Gerhard Krüss und Dr Hugo Krüss, Hamburg und Leipzig, 1891. Wolff's colorimeter is sold by the optical firm of Dr Hugo Krüss of Hamburg for 100 and 130 marks.

[2] Consult 'Ueber das Absorptionsspektrum des Eisenrhodanides und die zwischen Ferrisalzen und löslichen Rhodaniden stattfindende Reaktion,' at page 174 of the previously quoted work of the brothers Krüss.

A thorough treatment of Spectro-photometry will be found in the 2nd edition of Vol. I. of this work: a shorter account on a separate sheet accompanies this volume for the use of the reader.

Thus if we state that in the case of human saliva D=10—17, we imply that 1 cubic centimetre of human saliva converts in a period of five minutes from 10 to 17 cubic centimetres of standard starch emulsion to the achromic point, the temperature being 40° C.

Preparation of standard starch mucilage.

Five grammes of pure potato starch are well stirred up into a thin mud with 30 c.c. of water, and this is then poured in a slender stream into 470 c.c. of briskly boiling water. The mixture is stirred and allowed to boil for a few seconds. Standard starch mucilage should be used when fresh. After being kept for a few days it loses its opalescent appearance and slight mucilaginous consistency, and is then found to contain sugar.

Preparation of solution of iodine.

The solution of iodine is made by diluting one part of Liq. Iodi of the British Pharmacopœia with 200 parts of water.

Description of the actual process of diastasimetry.

10 c.c. of the standard starch mucilage are diluted to 100 c.c., and heated in a beaker over a flame to from 40°—45° C. A known volume, say 1 c.c., of the diastatic solution is then added to the mucilage, and the time noted. Then at intervals of a minute a drop of the *enzymosing* liquid is placed on a white slab with a drop of the iodine solution, and the time and result of last testing is noted. When the achromic point is reached the time is noted, and the interval from the commencement of the experiment is computed. If at the end of three minutes the mixture still gives the blue reaction of unaltered starch a new experiment is made, using two, three, or four times the volume of diastatic solution. If, on the other hand, the *achromic point* is reached in less than two minutes, a new experiment is made, using a smaller quantity of the extract.

Two or three experiments generally suffice to determine the quantity of diastatic solution required to bring the achromic point within a period ranging from two to ten minutes. A final control experiment enables the operator to fix the achromic point somewhere between four and six minutes. The accuracy of the method depends chiefly on the sharpness or precision with which the occurrence of the achromic point can be determined. If it occur earlier than two minutes the transition is too rapid for exact observation and record. On the other hand, if it occur later than fifteen or twenty minutes the transition is too gradual for precise limitation. The most satisfactory results are obtained when the achromic point falls between four and six minutes. The following example will serve as an illustration of the way in which the experiments are carried out, noted and expressed.

10 c.c. of standard starch mucilage + 90 c.c. of water
+ 0·1 c.c. of pancreatic extract, at 40° C.

Time.	Reaction with Iodine.
10.30 a.m.	Commencement of experiment.
10.31 „	Blue.
10.32 „	Violet.
10.33 „	Brown.
10.34 „	Yellowish-brown.
10.35 „	Pale yellow.
10.36 „	No reaction—achromic point.
6 minutes	

Mode of calculating the value D, from the results of the above experiments.

In the method of experiment which has just been described, the temperature and the volume of starch paste, are maintained constant, the volume of diastatic solution, and the time occupied by it in effecting the reaction are, however, varied. To obtain the value of D we must calculate, from the data obtained, what number of cubic centimetres of the standard mucilage would have been required, if the volume of diastatic solution had been 1 c.c. and the time five minutes.

Let v=volume in cubic centimetres of the diastatic solution,
n=time expressed in minutes,
D=diastatic value according to definition; then

$$D=\frac{10}{v}\times\frac{5}{n}.$$

Example. In the experiment previously cited to shew the usual course of events, 0·1 c.c. of pancreatic extract induced the *achromic* point in six minutes,

then $$D=\frac{10}{\cdot 1}\times\frac{5}{6}$$

$$D=83{\cdot}3,$$

that is to say, 1 cubic centimetre of the pancreatic extract used, would at the temperature of the experiment (40° C.), in the period of five minutes, convert 83 cubic centimetres of standard mucilage to the achromic point. Now, as the proportion of starch in the standard mucilage is always the same and known, viz. 1 grm. per 100 c.c., it is easy to calculate the weight of starch which has been brought to the achromic point; we have merely to divide D by 100 to obtain the weight of starch in grammes, or fractions of grammes; thus in the above experiment $\frac{83}{100} = 0·83$ grms., being the weight of dry starch converted.

In the method of diastasimetry which has been described, almost in the very words of its originator, Dr William Roberts. the reaction which is selected as the final reaction depends upon the disappearance of all dextrins which are coloured yellow by iodine, the solution containing only maltose and a dextrin which give no reaction with this reagent. Under the same circumstances as to temperature, the reaction proceeds with perfect regularity, and so as to admit of very accurate observations.

"Probably," as remarks Dr Roberts, "the most accurate mode of estimating the activity of a diastatic solution is to ascertain the amount of sugar produced when a given quantity of the solution is made to act on a given volume of a standard starch mucilage, for a fixed time, and at a given temperature. This method has already been recognized by Messrs Brown and Heron, in their paper, 'On the Transformation of Starch by Malt Infusions.' Kyeldahl has developed the method to a further point, and has used it to measure the comparative activity of malt infusions and of saliva."

The amount of sugar was determined in the experiments referred to by estimating the amount of a standard solution of a cupric salt which could be reduced by a known volume of the saccharine liquid.

The diastatic value may also be judged of in a similar manner by determining the rotatory power of the liquid which has been subjected to the action of the enzyme; in order to carry out this method, it would be necessary to separate the dextrins from the sugar formed, by the action of alcohol.

CHAPTER II.

GASTRIC DIGESTION.

SECT. 1. INTRODUCTORY. ON THE STRUCTURE OF THE STOMACH OF MAN AND CARNIVOROUS ANIMALS.

THE stomach consists of a muscular bag, the greater part of which is covered with a peritoneal investment which constitutes its serous coat, and which is lined by a mucous membrane in which are imbedded the glands whose mixed products give rise to the peculiar secretion, the gastric juice.

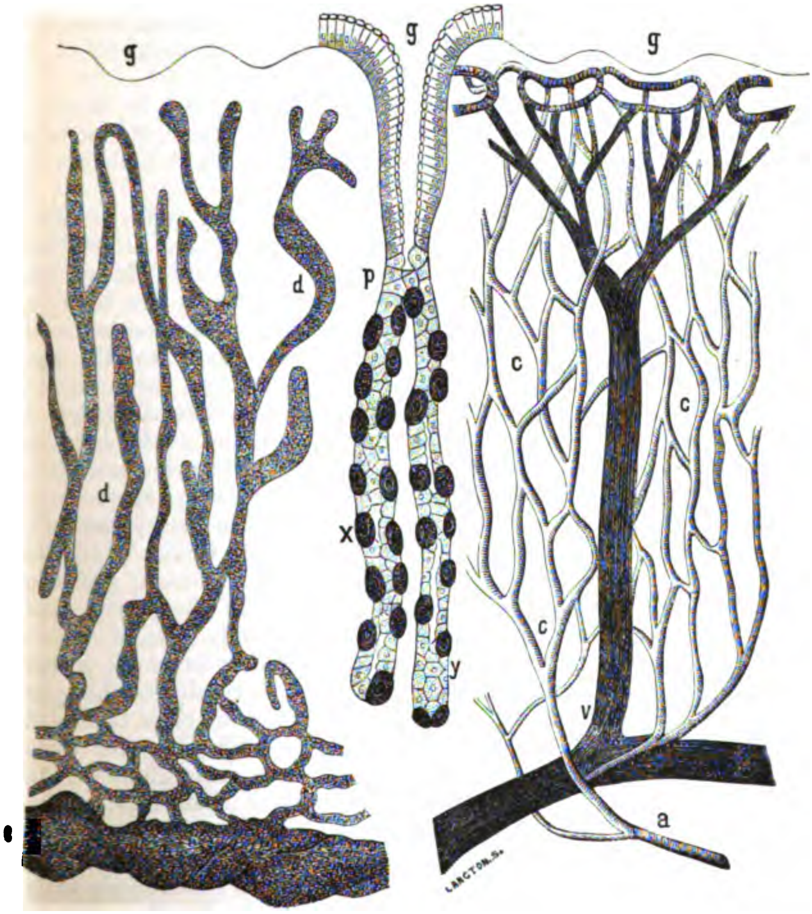
The mucous membrane of the stomach is a thin membrane, presenting prominent folds or *rugæ*, most abundant at its pyloric end, and which disappear when the organ is distended. With a magnifying glass the mucous membrane is seen to present innumerable pits or *alveoli*, which are separated from one another by intervening ridges, at the bottom of which are the open mouths of the gastric glands, which are tubular glands, simple or compound, occupying nearly the whole thickness of the mucous membrane.

In some animals, typically in the dog, the mucous membrane does not present one uniform appearance to the naked eye, nor is its structure identical in all parts. In the pyloric region it is less vascular, and appears thicker, though it is here much poorer in glandular structures than at the fundus.

In the stomach of all animals there are observed two sets of glands, which formerly used almost invariably to be classified by English writers, as (*a*) peptic, and (*b*) mucous glands, to indicate the view, then held, that the first secreted gastric juice, whilst the second merely secreted mucus. In the dog, the former are absent from the pyloric region, but occupy the mucous membrane of the fundus and curvatures; they are therefore often spoken of as the *glands of the fundus*. On the other hand, the other glands are spoken of as the *pyloric glands*. In animals where, as in the dog, these two structurally different regions of the stomach are observed, an intervening region, with transitional forms between these two sets of glands, has been described.

Cylindrical epithelium covers the alveoli and intervening ridges.

The whole mucous membrane of the stomach, with its depressed alveoli and the intervening ridges, is covered by cylindrical epithelium cells, similar to those of the intestinal tract. These epithelium cells are mucus-forming cells, the outer, free, portion of the cell being more or less completely transformed into mucin. The epithelial cells lie upon a basement membrane composed of apposed endothelium-like cells.



Schematic Representation of a Gland of the Fundus of the Stomach together with the lymphatics and blood-vessels of the Mucous Membrane. (Landois.)

g neck of the gland lined with cylindrical epithelium.
x and *y* secondary tubes lined with ovoid border cells and centric or chief cells.
a an artery of the mucous membrane breaking up into smaller branches and being in capillaries from the which arise the radicles of the vein *v*.
e deeper and *a* more superficial lymphatics of the mucous membrane.

The glands of the fundus. Peptic glands.

At the fundus of the stomach the mucous membrane appears thinner, but it contains a far greater amount of glandular elements than are found in the pyloric region. The individual glands are deeper, and they are separated from one another by a much smaller quantity of connective tissue.

The Peptic glands are usually arranged in groups of four or five. The open mouths at the bottom of the alveoli lead into ducts lined by cylindrical epithelium; "into each of these ducts open two or three tubes, the gland tubes proper."

In the gland tube we may distinguish, a somewhat constricted *neck*, and a main part the *body*, which increases in width as it proceeds towards the blind extremity.

The gland tube possesses a *membrana propria*, or basement membrane, upon the inner surface of which is placed the secreting epithelium, and outside of which are blood-vessels, lymphatics and nerves.

It has been said that the epithelium lining the duct common to several secreting tubes is columnar; in the glandular tubes themselves epithelium cells of two kinds are observed. Firstly, large ovoid cells, with oval nuclei, less numerous towards the blind end of the gland, are seen lying against the basement membrane and causing it in some places to bulge outwards. These are the *peptic cells*, properly so called, of the older English writers, the *border cells* (Belegzellen) of Heidenhain[1], the *delomorphous cells* of Rollet, the *oxyntic* cells of Langley; they do not form a continuous layer, but occur at intervals. The border cells are not distinctly granular in the fresh state, but become so on treatment with many reagents.

Situated internal to them and between them are cylindrical or cubical cells, the so-called *adelomorphous cells* of Rollet, which have been called 'Hauptzellen' or *chief cells* by Heidenhain, and which may most fitly be described as the *central* cells of the peptic glands. These central cells are recognized as essentially similar, both in structure and function, to the deeper columnar or more properly cubical cells which alone line the interior of the fundus of the pyloric glands. Heidenhain[2] points out, however, that the chief cells of the peptic glands present a coarse granulation which hides the borders of the separate cells, whilst the cells of the pyloric glands contain a much finer granular matter which allows of their borders being distinctly seen. On account of this and of some other differences the border cells and the pyloric cells cannot be regarded as being identical[3]. The lumen of the peptic glands is an exceedingly narrow canal, and contrasts with the much wider canal which penetrates to the depths of the pyloric glands.

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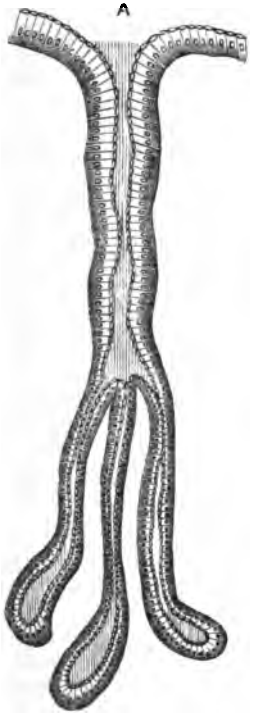
[1] Heidenhain, *Arch. f. mik. Anat.* VI. p. 368, 1870.

[2] Heidenhain, Hermann's *Handbuch*, Vol. v. p. 101, 1880.

[3] Langley and Sewall, 'Changes in Pepsin-forming Cells,' *Proc. Roy. Soc.* No. 194, p. 386, 1879; *Journ. of Phys.*, Vol. II. 299, 1879.

The pyloric glands.

Our knowledge of microscopical characters of the pyloric glands is chiefly due to Ebstein[1]. The characters of the pyloric glands are thus summarized, and compared with those of the peptic glands, by Dr Klein:—"The duct is proportionately very long; it amounts to half or more of the whole length of the gland: two or three tubes open into the duct by a very short neck, which represents the narrowest part of the gland: the body of the gland is branched into two or three tubes, which are wavy and convoluted; the lumen of the neck, but especially that of the body of the gland, is much larger than in the corresponding parts of the peptic gland; the lumen in the body of the former glands being many times longer than that of the latter. The epithelium covering the surface of the mucosa and lining the ducts in the pyloric region is exactly the same as in the rest of the stomach. The epithelium lining the neck and body of these glands is a continuation of that of the duct: but, as in the case of the peptic gland, so also here the cells are shorter and more opaque in the neck than in the body. In the latter the cells are fine, more or less transparent, columnar cells; in no part are there parietal cells[2]," &c.



SCHEMATIC REPRESENTATION OF A PYLORIC GLAND. (LANDOIS.)

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[1] Ebstein, *Arch. f. mik. Anat.* VI. p. 515, 1870.

[2] Klein and Noble Smith, *Atlas of Histology*, pp. 205 and 206, 1880.

SECT. 2. SOME HISTORICAL PRELIMINARIES. ON THE NATURE OF GASTRIC DIGESTION, AND THE CHARACTER AND PROPERTIES OF THE GASTRIC JUICE.

The views of the Ancients.

From an early period, full importance has been attached to the stomach as the chief organ engaged in the process of digestion. Hippocrates likened this process to one of coction, πέψις, the changes in the aliments being brought about mainly by the action of heat; an opinion which was afterwards advanced by Asclepiades, and by others who lived nearer to our own times. Some however assimilated the process to putrefaction; others explained it as entirely mechanical, foreshadowing the views which afterwards received fuller development at the hands of the disciples of the chemical and mathematical schools of physiology and medicine.

We may, following M. Milne Edwards[1], quote the following passage from Celsus as giving a summary of the views of writers antecedent to Galen.

"Ex quibus, quia maximo pertinere ad rem concoctio videtur, huic potissimum insistunt; et duce alii Erasistrato, teri cibum in ventre contendunt; alii Plistonico Praxagorae discipulo, putrescere; alii credunt Hippocrati, per calorem cibos concoqui; acceduntque Asclepiadis aemuli, qui, omnia ista vana et supervacua esse, proponunt: nihil enim concoqui, sed crudam materiam, sicut assumpta est, in corpus omne diduci[2]."

It is probable that in this passage Celsus attributes to Asclepiades views which he did not hold, and that the views of the fashionable physician of Rome were similar to and possibly suggested those adopted by Cicero, with whom he was on terms of intimacy, and who expresses himself concerning the nature and ends of the digestive process in the following most luminous manner.

"In alvo multa sunt mirabilia effecta;......est autem multiplex et tortuosa arcetque et continet, sive illud aridum et sive umidum, quod recipit, ut id mutari et concoqui possit; eaque tum astringitur, tum relaxatur atque omne, quod accipit, cogit et confundit, ut facile et calore, quem multum habet, et terendo cibo, et praeterea spiritu, omnia cocta atque confecta in reliquum corpus dividantur[3]."

The author appends the following literal translation of this interesting passage:—

"In the alimentary canal many wonderful acts are effected;......for it presents many folds and is tortuous, and encloses and retains that which it receives, be it dry or moist, in order that it may transform and digest it; at one time it contracts and at another relaxes, and it gathers and fuses together all which it receives, in order that by the heat of which it has much, by a process of grinding, and further by its exhalation (or spirit) all things being digested and prepared may be distributed throughout the rest of the body.'

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[1] Milne Edwards, *Leçons sur la Physiologie*, Vol. v. p. 252.

[2] A. Corn. Celsi, *Medicinae*, Libri octo, Lib. I.

[3] Cicero, *De Natura Deorum*, Lib. II. Cap. LIV. § 136 (page 52 of Mayor's Edition).

The views of the writers of the iatro-chemical school.

It was in the seventeenth century that the process of digestion was first looked upon as one akin to fermentation. Johan Baptista van Helmont (born 1577, died 1644), who in spite of the visionary nature of his views, has the merit of having been one of the earliest to shake the artificial fabric of medical dogma which had stood since the time of Galen, and which was so soon to fall through the efforts of the workers of the seventeenth century, first introduced the idea of *fermentation*, as explanatory of digestive action.

Under the influence of the *archæus*—as he termed the vital principle which presided over the processes of the organism, shaping its elements into the various forms of matter which compose its tissues and organs, there is generated in the stomach a ferment, whereby an acid is produced which brings about the solution of the food. Having attempted to prove, by argument, the insufficiency of the explanation that digestion was brought about through the influence of the heat of the stomach, v. Helmont dwells upon the properties of his 'fermentum acidum;'—

> 'Non est ergo calor digestionis author, sed est alia facultas quædam vitalis, quæ vere atque formaliter mutat alimenta. Eamque fermentorum nomine designavi. Sunt autem plurima fermenta in nobis, prout de digestionibus mox explicabo[1].'

That mere acidity was not sufficient to explain the digestion of the constituents of food was known to van Helmont, as is shewn by the following passages:

> 'Non est autem fermentum istud digestivum in sola aciditate aliquali situm. Neque enim acetum vel jus citri farinam fermentat: imo nec farina fermentata, est proinde fermentum stomachicum: sed hoc est acidum esurinum, stomachicum, specificum[2].'

Having tried to explain that this ferment must differ in different animals since the desire for and ability to digest different kinds of food is various, he defines his ferment as follows:

> 'Fermentum ergo digestivum, est proprietas essentialis, consistens in vitali quadam aciditate, ad transmutationes potens: ideoque et specificæ proprietatis[3].'

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[1] Van Helmont, *Ortus Medicinæ id est Initia Physicæ Inaudita Progressus medicinæ novus*. Edente Authoris filio Francisco Mercurio Van Helmont. Editio quarta. Lugduni, 1667. Refer to Chapter entitled 'Calor efficienter non digerit, sed excitative' (p. 126, paragraph 30).

[2] V. Helmont, *loc. cit.*, parag. 26.

[3] *Ibid.*, parag. 28.

F. De le Boé Sylvius. The idea that digestion was a process akin to fermentation was advocated by the celebrated F. De le Boé Sylvius (born 1614, died 1672), Professor of Medicine in the University of Leyden, one of the most celebrated medical teachers of his age, and the chief representative of the iatro-chemical school. Whilst v. Helmont had used the term fermentation to designate any chemical operation which appeared to him utterly inexplicable and essentially associated with vitality, Sylvius[1] by referring to alcoholic and acetous fermentations leads us to conclude, that these were types of the processes which he believed to occur in the alimentary canal. There is no evidence, however, of any original observations made by Sylvius in reference to gastric digestion, and on this as on all other matters he wrote so as to cause us to marvel that the crude dogmas and ridiculous jargon of the iatro-chemical school should even for a time have held sway in the medical world.

Descartes—Willis. Absurd though they appear to us, many of the doctrines of the iatro-chemical school received the support of such men as the great mathematician and philosopher René Descartes (born 1596, died 1650) and our own anatomist Thomas Willis (born 1621, died 1675).

Descartes believed that an acid of extreme potency, comparable to nitric acid, is generated in the stomach, as a result of a peculiar fermentation. Willis, who in spite of his thoroughly sound anatomical training, was a credulous adherent of the iatro-chemical school, also speculated upon the existence of an acid ferment in the stomach.

Grew. Although unconnected with the school to which reference has been made, the name of the observant Nehemiah Grew (born 1628, died 1711) must be mentioned amongst those who in the 17th century wrote concerning the nature of gastric digestion. Unlike those whose works have been referred to, Grew[2] wrote very briefly, but his writings mainly consisted, as was his wont, of the records of his own observations, though he could not resist the tendency of his age to speculation. He noticed the existence of glands in the mucous membrane of the stomach:

> 'By the joynt assistance of the glandulous and the nervous membranes the business of chylification seems to be performed. The mucous excrement of the blood supplied by the former, as an animal corrosive preparing, and the excrement of the nerves by the latter, as an animal ferment perfecting the work.'

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[1] F. D. Sylvii *Opera omnia*. Editio nova. Trajecti ad Rhenum, 1663. Refer to 'Disputatio Medica I. De Alimentorum fermentatione in ventriculo' (p. 11).

[2] Grew, *The Comparative Anatomy of Stomach and Guts begun, being several lectures read before the Royal Society in the year* 1676. London, printed for the author, 1681. See 'On the uses of the stomach of quadrupeds' (p. 26).

Researches of the iatro-mathematical school.

In strong contrast to the iatro-chemical school, the mathematical school of physiology was destined to render great and enduring services to science. Obviously, however, its methods were not suited to throw full light upon the essential processes of gastric digestion.

Ignorant of the fundamental facts of chemical science, the iatro-chemists had attempted, but in vain, to explain the functions of the body entirely by reference to chemical operations—the actions of acids and alkalies and fermentations, &c. The futility, nay the absurdity of their attempts had tardily forced themselves upon the minds of men, and there arose a school which attempted to explain all the phenomena of the body upon mechanical principles.

The muscular gizzard of birds had suggested the idea that gastric digestion did not consist in a maceration of the food under the influence of heat and moisture, as the father of medicine had imagined, nor of a fermentation such as v. Helmont and Sylvius had written about, but of a breaking-up of the particles of food by mechanical action. These muscular movements of the gizzard had been attentively studied by the philosophers of the Florentine Accademia del Cimento, amongst whom was the distinguished naturalist and physician, Redi (born 1626, died 1698), and their results had been placed on record.

The chief of the iatro-mathematical school was unquestionably Borelli (born 1608, died 1679), whose investigations[1] on the mechanical functions of the body must secure for him the respect and gratitude of physiologists of all ages. Borelli is said by Sprengel[2] to have advanced a purely mechanical theory of digestion. Such, however, was not the case. After describing the mechanical processes by which some animals comminute and, as he thought, digested their food, he refers to others in which another process is superadded:

> 'Hæc animalia fermento quoque validissimo carnes et ossa consumunt nec secus, ac aquæ corrosivæ metalla corrodunt, et dissolvunt. Talis porro succus corrosivus instillatur a glandulis corrosivis, quibus membranosa ventriculi substantia infarcta est, ut evidentissime observavi in ventriculo Delphini, cujus glandulæ admodum crassæ et prominentes sunt[3].'

Borelli thus more clearly enunciated the existence of a gastric juice and its relation to the glands of the stomach than any of his predecessors.

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[1] Borelli, 'De Motu Animalium.' *Opus Posthumum.* Romæ, 1681.

[2] 'Borelli expliquait bien plus mécaniquement les autres fonctions du corps. Nous avons déjà vu quelles étaient ses idées relativement à la force du cœur et au mécanisme de la respiration. Sa théorie de la digestion n'était pas moins conforme aux principes des iatro-mathématiciens. Il comparait l'estomac de l'homme à celui de différens oiseaux, et il en évaluait la force à un poids de mille trois cent cinquante livres.' Sprengel, *Histoire de la Médecine.* French edition, 1815. Vol. v. p. 142.

[3] Borelli, *Op. cit.* Vol. II. Prop. clxxxix. pp. 394 and 395.

The speculations of Pitcairn.

If a specimen of the absurd one-sidedness of certain of the followers of the iatro-mathematical school be sought for, it will be found in the essay of Dr Archibald Pitcairn, in which he sought 'without the assistance of a Dæmon or a Stygian Liquor,' to explain gastric digestion as entirely due to the triturating action of the coats of the stomach, the power of whose muscular walls he estimated as equal to 12951 pounds[1].

The views which prevailed as to gastric digestion in the middle of the 17th century.

In spite of the writings to which reference has been made, the opinions of scientific men were altogether divided on the question of gastric digestion until the middle of the last century. How far the process was due to the mechanical movements of the stomach, or the result of mere maceration, or to any special solvent agent, had yet to be determined, and the solution of these questions had to be obtained by patient observation of, and experiments upon, living creatures.

The discoveries of Réaumur.

. The French naturalist Réaumur (born 1683, died 1757), after many experiments[2] which had led to negative results, experimented upon a tame buzzard[3], which, like the hawk, owl, and other birds of prey, regurgitates those parts of its food, such as feathers, bones, &c., which are indigestible. To this bird, Réaumur administered small metallic tubes, containing various articles of food: the tubes were closed at one end and covered by muslin at the other, so as to preclude the possibility of trituration and yet permit of the gastric juice exerting its solvent action.

When meat was enclosed in the tube it was found to be digested after some hours; if the period which had elapsed since its introduction had not been sufficient, the surface was found softened, whilst the interior remained intact. Not merely was meat capable of complete digestion when exposed to the action of gastric juice in these tubes, which protected it from the mechanical action of the stomach, but even bone was softened and dissolved.

In order to obtain some of the solvent which effected these chemical operations Réaumur placed pieces of sponge in his tubes, and on their rejection was able to obtain from them a fluid of acid reaction, possessed of antiseptic properties, to which he called attention, but with which he was unable to effect digestion outside of the body.

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[1] *The Whole Works of Dr Archibald Pitcairn, &c.* Done from the Latin original by George Sewell, M.D. and J. I. Desaguliers, D.D. and F.R.S. 3rd edition. London, 1740. Refer to 'A Dissertation upon the motion which reduces the aliment in the stomach to a form proper for the supply of the blood,' pp. 106—138. Pitcairn was born in 1652 and died 1713. In 1691 he was appointed Professor of Medicine in the University of Leyden, an office which he held for a single year in order to return to Scotland, where he became a Professor in the University of Edinburgh. He is referred to as having been one of the teachers of Boerhaave.

[2] Réaumur, 'Sur la digestion des oiseaux' (premier mémoire), *Mém. de l'Acad. des Sciences*, 1752, p. 266.

[3] Réaumur, 'Sur la digestion des oiseaux (second mémoire): De la manière dont elle se fait dans l'estomac des oiseaux de proie.' *Mém. de l'Acad. des Sciences*, 1752, p. 461.

Réaumur, whilst convincing himself that in certain animals a chemical solvent plays an important part in the work of the stomach, was perplexed by the fact that vegetable food escaped the influence of the gastric juice, and was thus prevented from establishing that the essential of gastric digestion in all animals is the chemical action of the gastric juice upon certain of the constituents of food.

The researches of Stevens.

The discoveries of Réaumur constituted an important step in the discovery of the nature and processes of the gastric juice, and the subject was further elucidated by Dr Stevens, who in 1777 presented an Inaugural Thesis to the University of Edinburgh, entitled, 'De alimentorum concoctione.' He availed himself of the presence in Edinburgh of a Hungarian who was in the habit of swallowing stones and regurgitating them, a practice which he pursued as a means of gain by public exhibition. Stevens caused this man to swallow little silver balls perforated like a sieve, constructed so as to be filled with food and then closed by screwing. Dr Stevens found that the contained aliment was dissolved and sometimes completely disappeared, although protected from the influence of trituration. By constructing a silver ball with a median partition, the one half being more thickly studded with holes than the other, and filling both with food, he found that it was most readily dissolved in that division which contained most apertures. Additionally, Stevens obtained the gastric juice from the stomach of a dog, and he found that a piece of meat was digested by it outside of the stomach in eight hours, provided it were placed in a vessel exposed to warmth[1].

Spallanzani.

In the very year in which Stevens presented his thesis to the University of Edinburgh, the eminent Italian naturalist Spallanzani commenced his splendid investigations on digestion. These investigations[2], extending and corroborating those of Réaumur and Stevens, shewed conclusively that gastric juice was capable of effecting the same transformations when removed from the body, if the conditions for its activity were favourable, as in the stomach itself. He drew full attention to the antiseptic action of the gastric juice, and insisted that the process by which food is digested in the stomach is not akin to the 'alcoholic, acid and putrefactive fermentations.' Without knowing the cause of the acidity of the gastric juice, he yet recognised it, and asserted that the acid reaction ceases when digestion is completed.

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[1] Stevens, *De Alimentorum concoctione*, Edin., 1777.

[2] Spallanzani, *Expériences sur la Digestion de l'Homme et de différentes espèces d'Animaux...Avec des considérations*...par Jean Senebier, Ministre de l'Evangile, Bibliothécaire de la République de Genève. A Genève, 1783.

The researches which followed those of Spallanzani.

With Spallanzani closes the earlier history of the researches on the gastric juice. After him came Tiedemann and Gmelin[1], and Leuret and Lassaigne[2], who studied the acidity of the gastric juice.

Then followed the classical observations (1825—1833) of Beaumont[3], a surgeon in the United States' Army, made in connection with the case of Alexis St Martin, a patient in whom as a result of a gun-shot wound a gastric fistula had become established, which allowed both of the collection of gastric juice and of the observation of the processes which go on within the stomach.

Eberle[4] in 1834, announced the interesting fact that by acting upon the mucous membrane of the stomach by means of dilute hydrochloric acid, an artificial gastric juice can be obtained, with which food may be digested as by the natural gastric juice experimented upon by Spallanzani and Beaumont. Soon afterwards, Schwann[5] came to the conclusion that the gastric juice owed its peculiar activity to a principle which he denominated Pepsin, although he was unable to separate it.

These and all subsequent researches will be referred to again in detail in the sections which follow.

SECT. 3. METHODS OF OBTAINING GASTRIC JUICE.

We have seen that Réaumur and Spallanzani obtained small quantities of gastric juice by causing animals to swallow hollow perforated balls which contained pieces of sponge. The sponge absorbed gastric juice which was secreted under the stimulus of the foreign body thus introduced into the stomach, and by squeezing the sponge the juice was afterwards obtained.

Other experimenters, as Tiedemann and Gmelin, obtained small quantities of gastric juice by causing fasting animals to swallow insoluble bodies such as pebbles, and killing the animal shortly afterwards.

By these methods, observations of very great importance were unquestionably made; yet they were not sufficiently striking to convince all doubters, as may be proved by quoting a passage from a memoir of Schultz[6], a Professor in the University of Berlin, who in

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[1] Tiedemann u. Gmelin, *Die Verdauung nach Versuchen.* Heidelberg u. Leipzig, 1826.

[2] Leuret et Lassaigne, *Recherches sur la Digestion.*

[3] W. Beaumont, *Experiments and Observations on the Gastric Juice and the Physiology of Digestion.* Reprinted from the Plattsburgh edition, with notes, by Andrew Combe, M.D., Edin., 1838.

[4] Eberle, *Physiologie der Verdauung nach Versuchen.* Würzburg, 1834.

[5] Schwann, 'Ueber das Wesen der Verdauungsprocesse.' Müller's *Archiv*, 1836, pp. 90—138.

[6] Schultz, *De Alimentorum concoctione experimenta nova.* The author has not been able to verify this reference.

1834, pronounced himself in reference to the sound opinions of Réaumur and Spallanzani as follows;

> "Omnis Reaumurii atque Spallanzani opinio de succo gastrico nihil nisi vana hypothesis videtur, utpote cum effectus, quos succo gastrico imputaverunt soli potius salivæ tribuendi sint."

All doubts were however dispelled as to the essential part played by the gastric juice in digestion when it became possible for experienced observers actually to inspect the interior of the living stomach, to observe it under various conditions of rest and activity, and to follow *in situ* the digestion of aliments which they purposely introduced into its interior.

Gastric Fistulæ established by accident in Man.

The observations of Dr Beaumont on his patient, Alexis St Martin.

In the year 1822, Dr Beaumont, a surgeon in the military service of the United States, had under his care a young man, Alexis St Martin, who had been accidentally wounded by the discharge of a musket. The charge, consisting of powder and duck-shot, was received in the left side, the man being at a distance of not more than one yard from the muzzle of the gun. "The contents entered posteriorly, and in an oblique direction, forward and inward, literally blowing off integuments and muscles of the size of a man's hand, fracturing and carrying away the anterior half of the sixth rib, fracturing the fifth, lacerating the lower portion of the left lobe of the lung, the diaphragm, and perforating the stomach." After a most tedious and interrupted convalescence, the patient recovered: there remained, however, a large fistulous aperture some inches below, and a little to the outside of, the left nipple. This aperture, which measured about two and a half inches in circumference, at first allowed the contents of the stomach to escape, unless when occluded by compresses and bandages. Ultimately a prolapsed portion of the mucous membrane of the stomach came to act as a kind of valve which prevented the efflux of the gastric contents, but yet was easily depressed with the finger.

Having done all for St Martin which surgical skill and tact could accomplish, Dr Beaumont conceived the idea of utilising his remarkable patient for the purposes of science. Accordingly, taking the man into his own house as a servant, he undertook several sets of observations, only commencing the first of them, however, when St Martin's health had been completely restored. The first series of observations was commenced in the month of May, 1825, that is, nearly three years after the infliction of the injury, and this was followed intermittingly by other observations, of which the latest was performed in 1833. The results of Beaumont's observations were incorporated by him in a work published at Plattsburgh in that year.

The facilities afforded by Beaumont's patient for systematic observations on gastric digestion will be appreciated by a perusal of the following quotations:

"The valve, mentioned above, is formed by a slightly inverted portion of the inner coats of the stomach fitted exactly to fill the aperture. Its principal and most external attachment is at the upper and posterior edge of the opening. Its free portion hangs pendulous, and fills the aperture when the stomach is full, and plays up and down, simultaneously with the respiratory muscles, when empty."

"On pressing down the valve, when the stomach is full, the contents flow out copiously. When the stomach is nearly empty and quiescent, the interior of the cavity may be examined to the depth of five or six inches, if kept distended by artificial means, and the food and drinks may be seen entering it, if swallowed at this time, through the ring of the œsophagus. The perforation through the walls of the stomach is about three inches to the left of the cardia, near the left superior termination of the great curvature. When entirely empty, the stomach contracts upon itself, and sometimes forces the valve through the orifice, together with an additional portion of the mucous membrane, which becomes completely inverted, and forms a tumour as large as a hen's egg. After lying on the left side, and sleeping a few hours, a still larger portion protrudes, and spreads out over the external integuments, five or six inches in circumference, fairly exhibiting the natural rugæ, villous membrane, and mucous coat lining the gastric cavity. This appearance is almost invariably exhibited in the morning, before rising from his bed[1]."

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"*Mode of extracting the Gastric Juice.* The usual method of extracting the gastric juice, for experiment, is by placing the subject on his right side, depressing the valve within the aperture, introducing a gum-elastic tube of the size of a large quill, five or six inches into the stomach, and then turning him on the left side, until the orifice becomes dependent. In health, and when free from food, the stomach is *usually* entirely empty, and contracted upon itself. On introducing the tube, the fluid soon begins to flow, first by drops, then in an interrupted, and sometimes in a short continuous stream. Moving the tube about, up and down, or backwards and forwards, increases the discharge. The quantity of fluid ordinarily obtained is from four drachms to one and a half or two ounces (from about 14 to 56 grammes), varying with the circumstances and condition of the stomach[2]."

We shall have, in the sequel, to refer again and again to this very remarkable case, the observation of which proved so fruitful to science, merely remarking that no case of gastric fistula, whether established in man or the lower animals by accident, disease, or by the experimenter's knife, has ever afforded such admirable opportunities for study, for it occurred in a man of remarkable health, 'active, athletic and vigorous, exercising, eating and drinking, like other healthy and active people,' and it did not merely permit of the withdrawal of the gastric juice, and the introduction and withdrawal of catheters, thermometers, &c., but also of the ocular inspection of the resting and secreting organ.

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[1] Beaumont, *Op. cit.*, p. 23.
[2] *Ibid.*, p. 22.

Since this first, most successfully observed, case of gastric fistula, other cases have occurred of gastric fistulæ in the human subject, the study of some of which has led to important extensions of our knowledge. Especially does this remark apply to a case of gastric fistula established by M. Verneuil, in a boy in whom impassable stricture of the œsophagus came on as the result of swallowing a caustic alkali, and which has been subjected to elaborate study by Richet[1]; this case presented the valuable feature that the contents of the stomach were altogether unmixed with saliva.

Establishment of gastric fistulae in the lower animals.

The establishment of a gastric fistula as a result of an accident suggested the possibility of imitating the process by art. Accordingly a Russian and a French observer, Bassow[2], and Blondlot[3], almost simultaneously made the attempt; especially through the systematic and successful experiments of the second of these observers the procedure was carried to great perfection, and has been frequently repeated, many valuable facts having been discovered in this way. After Blondlot, Bardeleben[4], Bernard[5], Bidder and Schmidt[6], Schiff[7], Holmgren, Panum[8] and Heidenhain[9], have perfected and modified the method of establishing gastric fistulæ.

The following is a description of the whole operative procedure, which we quote in the words of Dr Lauder Brunton, F.R.S.

Establishment of a gastric fistula.

"The object of making a gastric fistula is two-fold; first to obtain gastric juice for examination; and second, to observe the process of secretion within the stomach itself.

"The method adopted by Bassow[10] was simply to make an incision in the abdominal parietes, to sew the stomach to the edge of the wound, and then to make an opening in the stomach itself. The fistula was plugged with a piece of sponge. It was, however, very liable to close, and was so made to allow the interior of the stomach to be observed. Blondlot prevented the wound from closing by placing in it a cannula, which was closed by a cork, so that the gastric juice and products of digestion might not be lost during the intervals between his observations.

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[1] Richet, *Le suc gastrique chez l'homme et les animaux.* Paris, 1878.

[2] Bassow, *Bulletin de la Société des Naturalistes de Moscou.* Vol. XVI. (1842).

[3] Blondlot, *Traité analytique de la digestion.* Paris, 1842, p. 202.

[4] Bardeleben, *Archiv f. physiol. Heilkunde,* Vol. VIII. (1849).

[5] A. Bernard, *Leçons de physiologie expérimentale.* Paris, 1856, p. 386.

[6] Bidder and Schmidt, *Die Verdauungssäfte,* p. 29 et seq.

[7] Schiff, *Leçons sur la physiologie de la digestion.* Paris, 1867. Vol. I. p. 15.

[8] Panum, 'Pepsin und Magenfistelanlegung.' Maly's *Jahresbericht,* Vol. I. p. 193.

[9] Heidenhain, 'Anlegung von Magenfisteln' in Hermann's *Handbuch der Physiologie,* Vol. v. Part i. p. 107 et seq.

[10] Bassow, *Bulletin de la Société des Naturalistes de Moscou,* Vol. XVI. (1842).

"This method, as improved by Bernard, is the one usually employed. Bernard's cannula consists of two tubes, each of which has at one end a broad flange. One tube screws into the other, so that the distance between the two flanges can be altered at will. This is effected by means of a key which fits on two projecting points in the inner tube and turns it round, while the outer one is held fast by the fingers. The advantage of this form over a simple tube with a shield at each end is, that the cicatrix of the wound often thickens in healing, and if the tube is not proportionately lengthened the outer plate presses on the skin and causes ulceration. The disadvantage of Bernard's cannula is, that it is too small to allow the interior of the stomach to be conveniently observed, and also, I think, that the edge of the wound comes into contact with the screw of the inner tube, and not with a smooth surface. These disadvantages may be readily obviated by increasing the diameter of the tube and the width of the flange, and adapting a key to the projecting points, by which the outer tube may be placed in the stomach and turned round as necessary. Such a cannula is represented in Fig. 6.

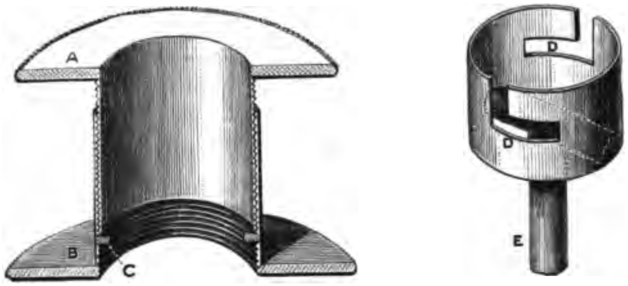

FIG. 6. CANNULA FOR GASTRIC FISTULA.

Operation for gastric fistula.

"Give the dog a hearty meal, so as to distend its stomach completely and make it lie close against the intestinal walls[1]. Anæsthetize the animal by chloroform[2], taking care that the vapour is mixed with a sufficient proportion of air. Lay it on its back on the table, shave off the hair from the epigastric and hypochondriac regions, and remove the hairs carefully by a sponge, so as to prevent the risk of their getting into the peritoneal cavity. Make a vertical incision about an inch and a half to one side of the *linea alba*, preferably to the left, and parallel to it, extending downwards from the lower edge of the costal cartilages to a distance somewhat less than the diameter of the flange of the cannula. Divide the muscles parallel to the course of their fibres. Tie every bleeding point before opening the peritoneum, so that no blood shall get into its cavity. Open the peritoneum on a director. Lay hold of the stomach with a pair of artery forceps at a point where there are not many vessels, and draw it forwards. Pass two threads[1] with a curved needle into the gastric walls at a distance from each other about equal to the diameter of the tube of the cannula, and bring them out again at a similar distance from the points where they were introduced. Make an incision into the gastric walls between the two threads, rather shorter than the diameter of the tube of the cannula. Put a pair of forceps, with the blades together, into the incision, and then dilate it by separating the blades till it is large enough to allow the cannula to be introduced. Push the cannula into the stomach so that the outer plate lies against the external abdominal wall. Tie the stomach to it by the threads, and then pass their ends through the edges of the wound in the abdominal wall in such a way as to fasten the stomach to it and at the same time to keep the cut edges in apposition. No other suture is required. Leave the cannula uncorked for at least half-an-hour after the operation is finished, for whenever the dog recovers from the chloroform it will vomit, and if the cannula be corked the fluid contents of the stomach are apt to be forced past the side of the cannula into the abdominal cavity. Feed the dog on milk for one or two days, and if the operation be performed in winter, keep it in a place warmed night and day. The day after the operation the edges of the wound will be much swollen, but the swelling will subside in a day or two. After the wound has begun to heal, the cicatrix may thicken, and the outer plate of the cannula begin to press too much on the rim, so that it ulcerates. If this should occur, the cannula must be lengthened by unscrewing the two flanges further apart. The cannula may be closed by an india-rubber stopper, or by a cork..................In order to collect the juice, let the animal fast for several hours, so that its stomach may be quite empty, but not for more than a day, as the mucous membrane would become covered with a thick coating of mucus. Let the assistant pat the dog, and keep it quiet; withdraw the cork from the cannula, and tickle the inside of the stomach with a feather tied to a glass rod. Put a small beaker underneath, so that the end of the rod rests on its bottom: the gastric juice will flow into it down the sides of the rod[2]."

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[1] Heidenhain, who has had great experience in the establishment of gastric fistulæ, prefers to operate upon fasting animals; his advice appears to the author to be unquestionably sound.

[2] It is preferable to anæsthetize the animal by a subcutaneous, or still better by an intravenous injection of morphia. Heidenhain, who adopts the latter plan, finds that a medium sized dog requires the injection of 4 c.c. of a 2 per cent. solution of morphia (=0·08 grm. of morphia).

Various experimenters differ on the question as to whether it is better to operate for gastric fistula on animals whilst fasting or with the stomach distended. Of recent experimenters Panum has declared himself in favour of operating when the stomach is full: Holmgren and Heidenhain, on the contrary, operate when the stomach is empty, the former distending the stomach with air by means of an elastic tube introduced through the œsophagus. The operation is said to be more successful when implicating the left than the right half of the stomach.

When it is stated, in conclusion, that the operation for gastric fistula should be performed with antiseptic precautions, and that in collecting the gastric juice some experimenters have found it useful to suspend the animal in a sling, the reader will have been placed in possession of the knowledge required by one about to make an investigation necessitating the establishment of gastric fistulæ.

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[1] Sutures of carbolised catgut should now be used.

[2] *Handbook for the Physiological Laboratory*, p. 477 et seq.

When a gastric fistula is established in a dog, the juice which flows from it is naturally mixed with saliva. To obviate this admixture some experimenters[1] established salivary fistulæ, so as to prevent the submaxillary and parotid secretions from entering the stomach.

SECT. 4. THE MORE OBVIOUS PHENOMENA ATTENDING SECRETION OF THE GASTRIC JUICE.

The Influence of the Nervous System upon it.

The fasting stomach is pale; it contains no gastric juice.

In man and many carnivorous animals the process of gastric digestion is one which, during health, only occupies a few hours. At its completion, the stomach is found quite empty, the pallid, uninjected mucous membrane being merely covered by a thin layer of mucus, which at the pyloric end usually has an alkaline, and at the cardiac end an acid reaction. There is no accumulation of gastric juice in the resting stomach, such as would occur if the gastric, like certain other glands, secreted continuously.

The observations made on this subject by Dr Beaumont on the man Alexis St Martin[2] are of far greater value than those made upon dogs with gastric fistulæ, for the man in spite of his fistula was in a thoroughly physiological condition, whilst a dog with a cannula *in situ* has *ipso facto* a constant stimulus acting upon the stomach. It is in this way that we explain the fact that large quantities of gastric juice have been found in the stomach of a dog with gastric fistula, though it had been fasting for 24 hours[3], and that some observers[4], drawing their conclusions merely from the case of dogs with gastric fistulæ, have asserted that the secretion of gastric juice like that of bile is a continuous one.

From the fact that during fasting the stomach does not contain gastric juice we should conclude that the saliva, which unquestionably is constantly being swallowed and passing into the stomach, is only capable, on reaching the gastric mucous membrane, of causing the secretion of a very small quantity of gastric juice, a conclusion which agrees with the observations of Heidenhain.

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[1] Bardeleben, and Bidder and Schmidt.

[2] These observations have been confirmed by the observation of other cases of gastric fistula in man. See Heidenhain, Hermann's *Handbuch*, Vol. v. p. 111 (footnote).

[3] Heidenhain, Hermann's *Handbuch*, Vol. v. (1880), p. 111.

[4] Braun, Eckhard's *Beitr. z. Anat. u. Phys.*, Vol. VII. (1876), p. 29.

A mechanical irritation, but still better, the presence of food, occasions a flow of gastric juice.

Whenever a foreign body, such as the bulb of a thermometer, is brought in contact with the interior of the stomach, the mucous membrane is seen to become turgid, and a flow of gastric juice is set up. The surface of the stomach is seen to be covered by "innumerable lucid specks," which seem to burst and "discharge a limpid, thin fluid over the whole gastric surface" (Beaumont). Whilst mechanical irritation will invariably occasion a flow of gastric juice, the best observers (Tiedemann and Gmelin, Heidenhain, &c.), confirm the statement of Beaumont, that by this means only a limited quantity of gastric juice can be obtained; the effect, as Beaumont surmised, appears in this case to be a purely local one.

When instead of a foreign body, solid aliment enters the stomach, the turgescence of blood-vessels of the organ is general and great, and gastric juice is poured out in quantity and continuously. The cause of the difference in the effect of purely mechanical stimulation and of stimulation by food is yet doubtful. Some light seems thrown upon it by an interesting observation of Heidenhain. This observer, as will be explained at length in the sequel, has succeeded in separating a portion of the cardiac end of the stomach from the rest of the organ, retaining, however, its vascular and nervous connections. By sutures, this portion of stomach is converted into a tube, blind at its inner end, but possessing an opening which is stitched to an incision in the abdominal wall. Thus a tube with walls composed of cardiac end of stomach can be obtained. Heidenhain observed that in a dog in which such a procedure has been successfully carried out, secretion of fluid from this stomach-tube came on from 15—30 minutes after food had been taken into the stomach, and continued to the end of digestion. If, however, a meal of indigestible substances were partaken of, secretion was much longer delayed, and would only occur when the animal began to drink, lasting even then but a comparatively short time. This observation, taken in connection with the fact that during digestion of digestible food gastric juice is abundantly and continuously secreted, whilst it is not so after mechanical irritation, points to the fact that the products of digestion when absorbed act as the essential stimuli to the secreting structures of the stomach (Heidenhain), and thus lead to the difference in behaviour of the stomach according as it is thrown into action by mechanical stimuli or by digestible food.

Facts which may be supposed to indicate that the

It was observed by Bidder and Schmidt that in dogs with gastric fistulæ in which salivary fistulæ had been established, and which had been starved, the sight of food caused an abnormal flow of gastric juice[1].

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[1] 'Sehr bemerkenswerth ist, dass bei nüchternen Thieren auch der blosse Anblick

secretion of the gastric juice is influenced by the nervous system.

Richet also observed[1], in the case of gastric fistula which he has submitted to elaborate investigation (that of Marcelin N.), that when the patient, who had an absolutely impassable stricture of the œsophagus, chewed savoury articles of food, there was, simultaneously with an abnormal secretion of saliva, a copious flow of gastric juice.

These facts would lead one at first to conclude that the gastric glands are under the closest influence of the nervous system, but, as is remarked by Heidenhain, who draws attention to them, the results observed may be explained by assuming an action exerted primarily on the muscular movements of the stomach, or upon the blood-vessels of the stomach.

Section of the pneumogastric nerves has long been known to influence in an important manner the functions of the stomach. It has been asserted, by some, that after division of these nerves the secretion of gastric juice, as well as the movements of the stomach, cease permanently; by others, that secretion of the gastric juice is stopped for a time, and then becomes re-established. The bulk of the evidence which is most to be relied upon clearly shews, indeed, that the pneumogastric nerves only affect gastric digestion by their influence upon the movements of the organ, and that after their division secretion of gastric juice of normal constitution takes place.

It has been similarly shewn that the sympathetic nerve-trunks connected with the stomach may be destroyed without stopping the secretion of gastric juice. The stomach therefore, unlike many secreting organs, is not known to be dependent on the control of secretory nerves passing to the organ from the great nerve-centres. It cannot, however, be regarded as definitely proved that there are no secretory nerves running from the central nervous system to the gastric glands. Since the gastric glands secrete when cut off from the central nervous system, we have to inquire whether this is brought about by a peripheral nervous mechanism or by a direct stimulation of the gland centre. The abundant nerve ganglia and nerve plexuses which are found in the submucous coat of the stomach may probably represent the secretory centres, and the secretory nerves which preside over the secretion of the gastric juice, but yet another possibility presents itself.

It is conceivable, argues Heidenhain, that just as in the vegetable Drosera the secretion of the digestive glands is brought about by mechanical irritation without the intervention of a nervous mechanism, so, in the animal stomach, secretion of gastric juice may follow the direct application of a stimulus to the secreting epithelium.

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von Nahrungsmitteln die Absonderung des Magensaftes zu vermehren vermag, wovon wir uns bei Thieren mit unterbundenen Speichelgängen vielfach überzeugt haben.' Bidder u. Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, p. 35.

[1] Ch. Richet, 'Recherches sur l'acidité du suc gastrique de l'homme...faites sur une fistule gastrique.' *Comptes Rendus*, Vol. LXXXIV. p. 410. *Journ. de Pharm. et Chimie*, Vol. XXV. p. 427. *Le suc gastrique chez l'homme et les animaux.* Paris, 1878.

SECT. 5. PHYSICAL AND CHEMICAL CHARACTERS OF THE GASTRIC JUICE.

Pure gastric juice, such as can be obtained by the stimulation of the mucous membrane of the stomach of an animal in which a gastric fistula has been successfully established, is a thin, usually colourless, though sometimes, as in the dog, yellowish liquid, possessed of a very acid reaction, and of a faintly acid mawkish taste, and of a peculiar though not easily defined odour.

It has a specific gravity, which varies between 1001 and 1010, the specific gravity varying in the same animal with varying conditions of the secretion.

When boiled, the gastric juice is not coagulable, but ceases to be active. When cooled to 0° C. the gastric juice of warm-blooded animals ceases to exert its peculiar digestive powers.

The gastric juice of man contains less than one per cent. of solid matters, of which about two-thirds are organic and one-third mineral.

The gastric juice may be kept for weeks and months without exhibiting any signs of putridity, and retaining its proteolytic activity. It possesses considerable antiseptic properties, as may be observed by moistening slightly putrid meat with the juice. This property is believed to be due to the free acid which it contains.

The essential constituents of the gastric juice summarized.

The essential physiological attribute of the gastric juice is the power of breaking down and dissolving a large part of the solid proteid aliments and converting them into so-called albumoses and peptones. This power depends upon the co-existence in the juice of an enzyme termed pepsin and an acid which has been shewn to be either free hydrochloric acid or a more complex conjugated acid formed by the union of hydrochloric acid with an organic body, which, however, if it exists, is readily dissociated with the evolution of hydrochloric acid. Neither pepsin nor hydrochloric acid are active alone, but a mixture of the two bodies, in the presence of a proper quantity of water and at a suitable temperature, acts essentially as the normal gastric juice. Whilst the enzyme pepsin is absolutely indispensable, the acid may be replaced by other acids and yet proper digestion will take place.

Besides the proteolytic ferment pepsin, the gastric juice in man and certain other animals contains a *milk-curdling* ferment, which we may term the curdling ferment or 'rennin' (Foster, Lea), and to which the name Chymosin has also been given by Deschamps[1]. Neither pepsin nor the rennet ferment have yet been isolated as pure chemical bodies, but our knowledge of their properties is derived from a study of solutions which contain them in a state of greater or less purity.

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[1] **Hammarsten,** *Lehrbuch d. physiolog. Chemie*, **Wiesbaden 1891, T. 153.**

Besides the enzymes we have mentioned, and more or less extraneous admixture with mucus, some fat, and organic products of digestion, the gastric juice contains alkaline chlorides, earthy phosphates, and iron. No experiments have been made to determine the presence or nature of any gases which it may hold in solution or feeble combination.

Various reactions exhibited by the gastric juice.

The following are some of the principal characters of pure gastric juice of the dog[1].

It is not coagulated on boiling, which however destroys its proteolytic properties. The acid juice is, according to some, coagulated by ferrocyanide of potassium, though the evidence on this point is not unanimous.

Concentrated mineral acids produce no turbidity, or precipitate.

Alkaline carbonates throw down a scanty precipitate, consisting mainly of earthy salts, carrying down with them, however, a portion of the organic matter, although the filtrate, when acidulated, still retains digestive powers.

Sodium chloride, when added to saturation, precipitates many of the albumoses which the juice contains, together with a large part of the ferments.

Mercuric chloride produces a precipitate, which contains a part, but not the whole, of the pepsin present.

Silver nitrate precipitates the chlorides and hydrochloric acid present, and likewise a part of the pepsin.

Lead salts, as lead acetate, form precipitates containing a great part of the pepsin. From this precipitate much of the pepsin may be separated by mere washing with water.

Alcohol produces a white precipitate, which, if the quantity of alcohol added be not excessive, slowly dissolves in water, yielding a solution which when acidulated with hydrochloric acid, digests actively. When a large excess of alcohol is however added to the gastric juice, the precipitate is said by Frerichs to lose for ever its digestive properties.

Results of analyses exhibiting the general composition of the gastric juice.

Before examining, in detail, the facts which are known concerning the ferments and the acid of the gastric juice, and their relation to the process of digestion, the attention of the reader is drawn to the following often quoted analysis, exhibiting the general composition of the gastric juice of the dog. No complete and at the same time reliable analysis of the gastric juice of man is available[2], however there is no reason for supposing that the secretion in man is sensibly different from that of the dog.

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[1] Frerichs, Article 'Verdauung' in Wagner's *Handwörterbuch*, Vol. III. p. 785.

[2] There is a constantly quoted analysis of the gastric juice of a woman, made by C. Schmidt, which the author cannot admit as satisfactory inasmuch as the free acid which it reveals is at least ten times below that which is now known to be present in healthy human gastric juice.

COMPOSITION OF THE GASTRIC JUICE OF THE DOG, OBTAINED WITHOUT ADMIXTURE WITH SALIVA (THE MEAN OF TEN ANALYSES BY C. SCHMIDT).

Water in 1000 parts	973·062
Organic matters (including peptones, pepsin, mucin)	17·127
Free HCl	3·050
NaCl	2·507
KCl	1·125
NH_4Cl	0·468
$CaCl_2$	0·624
$Ca_3\ 2(PO_4)$	1·729
$Mg_3\ 2(PO_4)$	0·226
$FePO_4$	0·082

SECT. 6. ARTIFICIAL GASTRIC JUICE.

It has been stated that the most striking property of the gastric juice is its power of dissolving and digesting solid proteid bodies, providing the process be carried on at a favourable temperature, that is from 20°—40° C.; it has also been stated that this property depends upon the presence of an enzyme called pepsin and a free acid, neither of these being capable of acting, in its characteristic manner, independently of the other.

Eberle was the first to shew that the solid mucus which can be scraped from the surface of the mucous membrane of the stomach undergoes solution under the influence of dilute acids. And he thus prepared an artificial gastric juice which possessed the power of digesting suitable substances at a proper temperature[1]. Eberle likewise found that acid in which mucous membrane of the stomach had been digested possessed powers of dissolving proteids. Eberle however fell into the great error of supposing that mucus was the essential proteolytic agent, and actually asserted that with mucus taken from other organs than the stomach, as for example with nasal mucus, digestive liquids could be prepared.

The observations of Eberle were first repeated by Johannes Müller, shortly afterwards by J. Müller and his pupil Schwann[2], and they brought to the work a perspicuity and a scientific acumen which were singularly absent in the first observer. It was Schwann[1] who pointed out how erroneous was the view of Eberle that mucin was the principle which conferred proteolytic powers upon an acid infusion of the mucous membrane of the stomach, by shewing that the active principle held in solution in artificial digestive juice prepared by the action of a dilute acid upon the gastric mucous membrane, had properties which are different from those of mucin, and further that by acting upon mucus obtained from other organs than the stomach, a true digestive liquid cannot be obtained.

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[1] Eberle, *Physiologie der Verdauung nach Versuchen.* Würzburg, 1834. See at page 156 the following passage: "Magensaft von Thieren gewonnen, bewirkte die Chymification mehrerer Speisen in der Wärme auch ausser dem Magen, und nach meinen eigenen Versuchen gelingt endlich noch die Chymification der Nahrungsmittel durch den künstlich bereiteten Magensaft in der Wärme vollständig."

[2] J. Müller u. Th. Schwann, 'Versuche über die künstliche Verdauung des geronnenen Eiweisses.' Müller's *Archiv*, 1836, p. 66.

Methods of preparing artificial gastric juice.

1. The stomach of a pig is opened, emptied of its contents, and then the surface cleaned with a wet sponge ('running water' will dissolve out a considerable part of the pepsin). The mucous membrane is removed from all but the pyloric end of the organ. It is then freed from a portion of the water which is adhering to it by pressure between dry cloths, and minced. The finely divided mucous membrane is then placed in two or three litres of dilute hydrochloric acid containing from six to ten c.c. of strong HCl per litre, and the mixture is digested in the incubator at a temperature of 35°—45° C. for a period varying from a few hours to a day. If sufficient fluid be present and the mixture now and then shaken, all ought to be dissolved in a few hours, leaving but a small quantity of brownish flakes and some mucus undissolved. The liquid is filtered through paper, and then may be kept for several months, without undergoing decomposition, and retaining active proteolytic properties.

Artificial gastric juice prepared in this way is very energetic in its action, and forty or fifty cubic centimetres added to 200 c.c. or 300 c.c. of 0·1 per cent. HCl solution, will be usually found to furnish a highly active digestive fluid. Such a juice does not, however, contain merely acid and pepsin, but considerable quantities of albumoses and peptones.

2. The following method was recommended long ago by Kühne[2] for obtaining a juice possessed of considerable activity and yet containing but small quantities of peptones. Open the stomach soon after death, empty it, and wash it thoroughly in cold water. Then scrape the surface with a blunt instrument so as to remove a layer of mucus mixed with epithelium cells. The matter thus removed is rubbed up with pure quartz sand, or glass-powder, and cold water, which dissolves the pepsin which the mixture contains. On filtering, an opalescent liquid is obtained which, when acidulated so as to contain 0·1—0·2 per cent. of HCl, possesses powerful digestive activity.

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[1] Schwann, 'Ueber das Wesen des Verdauungsprocess.' Müller's *Archiv*, 1836, p. 90.

[2] Kühne, *Lehrbuch d. physiologischen Chemie.* Leipzig, 1868, p. 38.

3. Of all methods yet suggested for the preparation of an active artificial gastric juice, the following, suggested by Kühne and Chittenden, furnishes the most active preparation, and also one in which the proportion of extraneous products is in smallest amount. The mucous membrane, from the cardiac region of several pigs' stomachs, say five or six, is reduced to a fine state of division and is then digested for fourteen days at 40° C. with from two to three litres of 0·5 per cent. hydrochloric acid.

At the end of this time in presence of so large a quantity of pepsin and acid, all but traces of the so-called albumoses have been converted into peptones, which are in solution together with pepsin, although there remain undissolved a small proportion of foreign matters, nucleins, anti-albumid, &c.

The liquid is filtered and saturated with powdered ammonium sulphate. This salt in addition to its power of precipitating any albumoses which may be present, throws down the whole of the pepsin. The precipitate is collected on a filter, washed with saturated solution of ammonium sulphate, and is then dissolved in 0·2 per cent. hydrochloric acid.

To the acid solution is added 0·25 per cent. of thymol, and it is then dialysed in running water, until the whole of the ammonium sulphate has been removed.

On opening the dialysing tubes a precipitate is found, which is soluble in 0·2 per cent. HCl, and furnishes a very active gastric juice. The filtrate also when acidulated so as to contain 0·2 per cent. of HCl, furnishes an intensely active gastric juice[1].

4. Take a glycerin extract of mucous membrane of the stomach and mix with dilute hydrochloric acid containing 0·2 per cent. HCl.

5. A solution of pepsin prepared by methods afterwards to be described, may be added to dilute hydrochloric acid of suitable strength, so as to furnish an artificial gastric juice of great purity.

Strength of hydrochloric acid for artificial gastric juice.

We have recommended above that dilute hydrochloric acid containing 0·1 or 0·2 per cent. of HCl should be used, for it resulted from the observations of Brücke that, *cæteris paribus*, pepsin acts most energetically on many proteids if present in a fluid containing approximately this quantity of hydrochloric acid; the most favourable strength for the solution of fibrin being 0·086—0·088 per cent., whilst for coagulated white of egg it is as high as 0·12 or 0·16 per cent. It would appear however from the observations of Kühne and Chittenden that the digestive process is, in some cases at least, much more active if a stronger HCl, even up to 0·5 per cent., be employed.

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[1] Kühne and Chittenden, 'Ueber die Peptone.' *Zeitschrift für Biologie*, Vol. XXII. p. 423. And in Studies from the Laboratory of Physiological Chemistry of Yale University, edited by R. H. Chittenden, Ph.D., Vol. II. (1887) p. 18 and Chittenden and Bolton, 'Egg-Albumin and Albumoses.' *Stud. from Lab. of Phys. Chem. of Yale Univ.* Vol. II. p. 185.

Hydrochloric acid may be replaced by other acids in the preparation of artificial gastric juice.

Hydrochloric acid may be replaced by other dilute acids in the preparation of artificial gastric juice, as by nitric acid, tribasic phosphoric acid, lactic acid, &c. It appears that sulphuric, acetic, oxalic and tartaric acids act more feebly[1].

Determination of the activity of an artificial gastric juice.

We usually determine that an artificial digestive juice is possessed of proteolytic activity by placing it in an incubator, or in other ways maintaining it at a temperature favourable to peptic proteolysis (35°—50°C.), and then adding to it (*a*) a flocculus of well washed fibrin, preferably of fibrin which has been previously swollen by digestion in cold dilute solution of hydrochloric acid (·1 per cent.): (*b*) thinly cut slices of coagulated white of egg: or (*c*) boiled white of egg finely pounded in a mortar and pressed through a fine sieve: and observing the time occupied in the solution of the proteids used. A full description of the methods of determining the relative amounts of pepsin in different solutions will be given in the sequel.

Chemical Agents which influence Peptic Digestion[2].

All chemical agents which precipitate pepsin arrest digestion by it, and generally the salts of the heavy metals exert this action, as lead acetate, copper sulphate, mercuric chloride and alum. Neutral salts of the alkalies and alkaline earths, as sodium chloride and sulphate, magnesium sulphate, and potassium iodide hinder peptic digestion. Arsenious acid is apparently without action. Hydriodic and hydrobromic acids hinder peptic digestion. Sulphurous acid arrests it. Hydrocyanic acid has but very slight action.

Whilst tannic acid arrests digestion, some of the organic acids which have most powerful action on organized ferments, hinder the action of the peptic enzyme but little. Thus in small quantities carbolic acid does not hinder digestion; in medical practice it is indeed found that carbolic acid often not only checks abnormal processes of fermentation going on in the stomach, but that when administered together with pepsin, it actually seems to aid this body.

Salicylic acid in large doses interferes with peptic digestion, though according to Kühne the pepsin is not destroyed even by digestion for several days with large quantities of salicylic acid. In diminishing the rapidity of peptic digestion salicylic acid is, however, certainly more powerful than carbolic acid.

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[1] See many authorities quoted by Maly on this subject, Hermann's *Handbuch*, Vol. v. Part ii. (1881), p. 72.

[2] The author has derived his information on this subject from Prof. Maly's article in Hermann's *Handbuch der Physiologie*.

SECT. 7. AN ACCOUNT OF THE ATTEMPTS TO SEPARATE PEPSIN, AND TO ESTABLISH ITS CHARACTERS.

Pepsin.

Eberle was the first, as we have seen, to shew that the mucous membrane of the stomach undergoes solution under the influence of dilute acids, and he described a mode of obtaining an artificial gastric juice which possessed the power of digesting suitable substances at a proper temperature. His experiments were, however, concerned rather with the behaviour towards chemical reagents of his artificial gastric juice than with the study of its real digestive activity, the nature of which he much misunderstood. Schwann, we have also seen, almost immediately afterwards took up the investigation of artificial gastric juice. He pointed out that the mucous membrane of the stomach alone was capable of yielding an artificial gastric juice; that it did not, as Eberle had thought, share this property with other mucous membranes, and he set about trying to isolate the principle which conferred upon dilute acids the property of dissolving certain of the food constituents.

Schwann's attempts to isolate the digestive ferment.

The mucous membrane of the stomach was digested in water, and the aqueous solution was treated with ferrocyanide of potassium, so as to precipitate the proteids present in the solution. The fluid was filtered, and the filtrate was neutralized with potassium carbonate; it was then precipitated with a solution of corrosive sublimate. The precipitate produced by this body was suspended in dilute hydrochloric acid and decomposed by means of sulphuretted hydrogen. The solution filtered from sulphide of mercury possessed intense proteolytic activity. To the proximate principle present in the mucous membrane of the stomach, which he in some degree had separated by his process, Schwann gave the name of Pepsin, without however laying any pretence to having isolated it.

Wasmann's method of preparing pepsin.

By a modification of Schwann's method, Wasmann[1] soon after succeeded in obtaining a soluble solid pepsin, possessed of very intense activity.

The mucous membrane from the fundus of a pig's stomach, was carefully dissected off and treated with water at 30°—40° C., and after some hours the liquid was poured off, the mucous membrane being thereafter treated again and again with cold water. The united watery liquids were precipitated with lead acetate or mercuric chloride, the precipitate containing lead or mercury compounds of proteids, entangling peptones, was collected, suspended in water, and decomposed by means of sulphuretted hydrogen, and from the filtrate after concentration, pepsin (mixed with proteids) was precipitated by means of alcohol.

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[1] Wasmann, *De digestione nonnulla*, *Diss. Inaug.*, Berolini, 1839. The author has not been able to see this dissertation. The account of Wasmann's pepsin given in the text is taken almost *verbatim* from Maly, 'Chemie der Verdauung,' Hermann's *Handbuch*, Vol. v. Part ii. (1881), p. 44.

The precipitated flocculi when dried yielded a *yellow*, gum-like matter. Acids caused a turbidity in the solution of this pepsin, metallic salts produced a precipitate.

According to Wasmann the proteolytic action of his pepsin was so great that one part in 60,000 of water, when acidulated, dissolved coagulated albumin in from six to eight hours.

By Wasmann's method, as by all other methods yet suggested, it is impossible to prepare pure pepsin—though unquestionably, assuming his assertions to be correct, his method yielded him an extraordinarily potent product.

Brücke's[1] method of isolating pepsin.

The mucous membrane of the pig's stomach is separated from the subjacent muscular coat, and after careful washing and removal of the adhering water by pressing between blotting-paper, is finely divided, preferably in a mincing machine. The mass is then digested in a 5 per cent. solution of tribasic phosphoric acid, at a temperature of 35° C., until nearly the whole is dissolved. The solution contains all the pepsin in solution, together with large quantities of parapeptones and peptones. The acid fluid is *almost* neutralized by the addition of lime-water, which causes a precipitate of $Ca_3(PO_4)_2$. This precipitate carries down with it much of the pepsin previously dissolved, whilst a considerable portion of the parapeptones and the whole of the peptones are left in the solution. The gelatinous precipitate is carefully washed with water, pressed, suspended in water and HCl added until it just dissolves. The solution is then poured little by little to the bottom of a tube containing a saturated solution of cholesterin made by dissolving that body in a mixture of four parts of alcohol and one part of ether. When the slightly acid aqueous solution comes in contact with the ethero-alcoholic liquid it produces a precipitate of cholesterin; this precipitate is repeatedly shaken up with the liquid in which it is produced. The cholesterin which has carried down with it mechanically a part at least of the pepsin originally present, is collected on a filter and is washed first with water, then with acetic acid, and lastly again with water, until the wash-waters are no longer acid and give no turbidity when treated with silver nitrate.

The moist cholesterin is now shaken up in a stoppered bottle with pure ether. The liquid in the bottle then separates into two layers, the upper of which is composed of an ethereal solution of cholesterin, the lower of water (which had adhered to the cholesterin) holding pepsin in solution. The ethereal layer is separated from the latter, which is shaken again and again with ether, until all traces of cholesterin are removed. The aqueous solution is then found to be slightly turbid, but on filtration may be obtained perfectly clear. The filtered liquid, when acidulated, possesses proteolytic activity. When allowed to evaporate spontaneously, it leaves a greyish, amorphous, non-hygroscopic, nitrogenous body, soluble with some difficulty in water, but more readily soluble in dilute acids.

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[1] Brücke, *Vorlesungen*, Vol. II. p. 300.

There is no reason to suppose that the substance obtained by this method is a definite chemical individual. It has been alleged in its favour that it yields the digestive enzyme pepsin in a purer condition than that in which it could be obtained by older methods. The process of preparation is however tedious in the extreme and very costly, and it has made way for other methods which furnish us with much more active solutions of pepsin, though still not of a pepsin which we can consider to represent the pure enzyme. This method has not in the hands of later observers given satisfactory results.

An aqueous solution of Brücke's pepsin is not precipitable by platinum tetrachloride, by mercuric chloride, by lead acetate, neutral or basic, by tannic acid, by iodine, or by concentrated nitric acid. The solution only exhibits in a faint manner the xanthoproteic reaction. According to Brücke it is precipitated by neutral and basic lead acetate and made cloudy by solution of platinum tetrachloride; though Krasilinikow, in Brücke's laboratory, by dialysing the solution, got rid of the platinum reaction, but not of the lead acetate reaction.

v. Wittich's method of preparing a solution of pepsin in glycerin.

Pepsin, as was discovered by v. Wittich[1], shares the property possessed by the majority of enzymes, of being soluble in glycerin. In order to prepare a glycerin extract of pepsin, the finely divided and cleansed mucous membrane of the fundus of the stomach may be placed for eight or ten days in concentrated glycerin. On subsequently straining and filtering, a glycerin solution of pepsin of considerable activity is obtained.

A better way of proceeding is to place the mucous membrane for twenty-four hours in water, and then to dehydrate the finely divided mucous membrane by placing it for twenty-four hours in an excess of 80 per cent. alcohol, filtering, driving off the alcohol which adheres to the tissue by evaporation in air, comminuting the dried residue still further, and then adding to it its own weight of glycerin. After some days the glycerin is strained off and replaced by a fresh quantity, the process being repeated several times.

From the glycerin extract of pepsin the impure ferment may be obtained in a solid form by adding a large excess of absolute alcohol, which precipitates it. This impure pepsin may be further precipitated by having recourse to the method described in the next paragraph. It may be noted that much less pepsin is obtained from a mucous membrane which has been treated with absolute alcohol, than from one which has been simply dried or taken fresh.

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[1] v. Wittich, Pflüger's *Archiv*, Vol. II. p. 193, and Vol. III. p. 193.

Methods of purifying pepsin based upon its non-diffusibility.

Pepsin in solution appears to be absolutely indiffusible through parchment-paper. Given, then, an acid solution such as artificial gastric juice, which contains traces of albumoses, small quantities of peptones and pepsin, the first-named bodies may be got rid of by long-continued dialysis and filtration, a dilute aqueous solution of pepsin ultimately remaining in the dialyser[1]. The non-diffusibility of pepsin from a solution containing it into pure water was first pointed out by v. Wittich[2], who however stated that when the dialyser was surrounded by dilute hydrochloric acid (of 0·2 per cent.), pepsin did diffuse. Hammarsten[3] afterwards shewed that the latter statement was incorrect, and established the fact of the absolute indiffusibility of pepsin into either neutral or acid solutions.

In order to prepare a solution of pepsin by the aid of dialysis, we may proceed in various ways, as (*a*) an artificial gastric juice, made in the way previously described, is subjected to dialysis for a period of several days. (*b*) Having followed out Brücke's method for the preparation of pepsin so far as the solution of the pepsin-containing precipitate of calcium phosphate in dilute hydrochloric acid, this solution, having been neutralised, may be subjected to prolonged dialysis (Maly). (*c*) Having precipitated by means of alcohol impure pepsin from a glycerin extract of the fundus, the precipitate is dissolved in dilute hydrochloric acid (containing 0·2 per cent. of HCl), and the solution is then dialysed.

In all these cases it is best to place the dialyser in a running stream of water, the actual form of dialyser being that suggested by Kühne, in which a tube of parchment-paper contains the solution to be subjected to the process of diffusion. As these tubes are now very frequently made of parchment-paper which is not perfect, it is very convenient to adopt the following plan, which has been communicated to the author by Mr Benger, of Messrs Mottershead and Co., of Manchester, and which answers admirably. A sheet of De la Rue's parchment-paper is soaked in water, and when thoroughly pliable is drawn together so as to form a bag into which the liquid to be dialysed is poured. The bag is then tied up tightly and suspended in a stream of running water. If it be desired to remove the last traces of diffusible substances, such as peptones, from the solution of pepsin, the process of dialysis must be carried on for a period of from eight to fourteen days; and in order to avoid putrefactive changes thymol may be added to the liquid which is being dialysed or the acid which has diffused away must from time to time be restored (Hammarsten)[1]. It is obviously desirable to keep the bag or tube in a state of continuous or rythmically interrupted agitation by the aid of a motor, as in Lea's apparatus figured at Page 46.

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[1] Dr Krasilnikow in 1864 was, according to Hammarsten, the first to make use of dialysis for the preparation of pure solutions of pepsin.

[2] v. Wittich, 'Das Pepsin und seine Wirkung auf Blutfibrin.' Pflüger's *Archiv*, Vol. v. p. 435.

[3] Hammarsten, 'Ueber die Indiffusibilität des Pepsins.' Maly's *Jahresbericht*, Vol. III. (1874), p. 160.

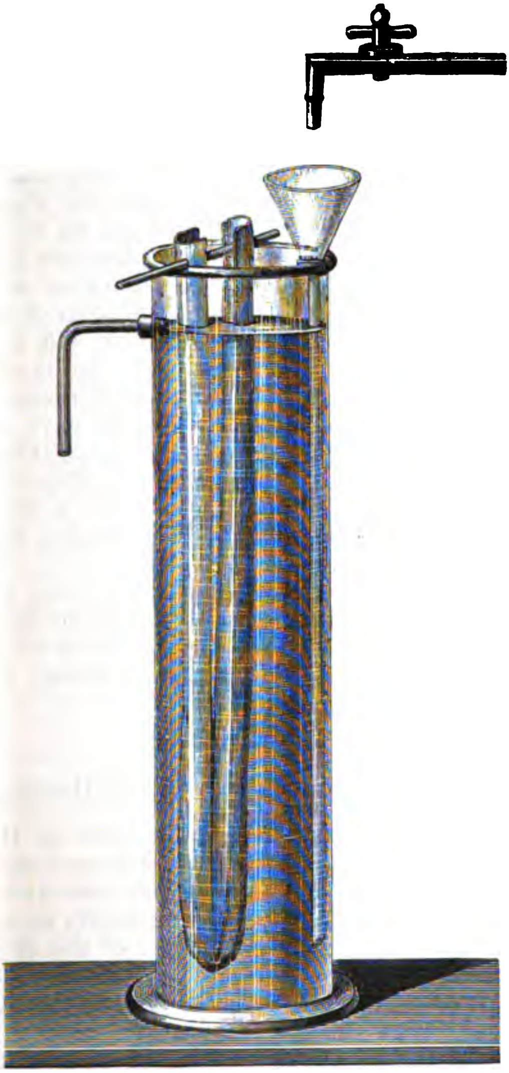

FIG. 7. KÜHNE'S ARRANGEMENT FOR DIALYSING IN A CONTINUOUS STREAM OF WATER.

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[1] The non-diffusibility of pepsin was also shewn by Wolffhügel in a paper entitled 'Ueber Pepsin und Fibrinverdauung ohne Pepsin.' Pflüger's *Archiv*, Vol. VII. (1873), p. 188.

Commercial preparations of pepsin.

Various preparations have been sold in commerce under the name of Pepsin. Officinal pepsin has in many cases consisted merely of the dried mucous membrane of the stomach of the pig reduced to powder and mixed with starch or milk-sugar. Some has consisted of a product made essentially by Wasmann's method (see p. 85) mixed with large quantities of starch or sugar. One of the most active of the preparations of commerce is made by a method devised by Schaffer, and which rests upon the following facts. If to artificial gastric juice sodium chloride be added in large quantities, the albumoses which are held in solution are precipitated and rise to the surface as a scum, which may be ladled off; this scum is however rich in pepsin, which is mechanically retained by the albumoses as it is by the calcium phosphate precipitate in Brücke's process of pepsin preparation. The scum is collected and preserved, and is either dried and afterwards powdered, or it is mixed, whilst yet moist, with sugar of milk. In both these forms it has been sold in commerce.

Various very active preparations of pepsin have of late made their way in commerce, as for instance, Jensen's 'Crystal Pepsin,' Liebreich's 'Pepsin Essenz,' Benger's 'Liquor Pepticus,' and Bullock's 'Acid Glycerin of Pepsin,' which last is a glycerin extract of extraordinary and remarkably uniform strength, and possessing scarcely any other taste than that of glycerin.

Having described the various modes of obtaining pepsin and its preparations, it is necessary to discuss the nature of the acid or acids of the gastric juice, before undertaking the study of gastric digestion, from which alone we can derive any satisfactory information as to the properties of pepsin.

SECT. 8. THE ACID (OR ACIDS) OF THE GASTRIC JUICE.

It was pointed out in the historical account of the earlier researches on digestion that many of the older writers supposed gastric digestion to be due to the secretion of an acid corrosive fluid.

The fact that the gastric juice was *essentially* an acid fluid was not admitted until nearly the first quarter of the present century had passed. It is true that some observers, as Carminati, Brugnatelli, Viridet, Werner[1], Montégre[2], had experimentally determined that in particular cases the gastric juice had an acid reaction, but their observations were not supported by those made by Spallanzani and others[3]. Moreover certain of the evidence adduced in favour of the acidity of the gastric juice was not sufficient, as we now know, to prove the fact; as for instance that milk is curdled by the secretion of the stomach, a phenomenon now known to be independent of an acid reaction.

[1] Carminati, Brugnatelli, Viridet, Werner, quoted by Tiedemann and Gmelin in *Die Verdauung nach Versuchen*, Vol. I. p. 147.

[2] Montégre, *Sur la Digestion de l'Homme*. Paris, 1804.

[3] See Magendie, *Précis élémentaire de Physiologie*, T. II. p. 11.

Prout's discovery of hydrochloric acid in the gastric juice.

In 1824 Dr Prout[1] published his investigations on the nature of the acid of the gastric juice, which were conducted upon the watery solution of the acid contents of the stomachs of animals killed during digestion, and upon acid vomited matters of man. Dr Prout did not merely determine the acidity of these liquids by ascertaining the amount of alkali required to neutralize them, but he shewed that besides containing considerable quantities of chlorides, the acid contents of the stomach contained hydrochloric acid which could be separated by distillation.

These observations were confirmed by Children[2] and by Braconnot[3]. Tiedemann and Gmelin[4], though admitting that they had in certain cases discovered hydrochloric acid in the contents of the stomach, were of the opinion that Prout had been in error in considering it as the only acid present, as they had found both acetic acid and butyric acid.

Beaumont's observations on St Martin conclusively reconciled the discrepant statements of previous observers by shewing that whilst the gastric mucous membrane may have a neutral or alkaline reaction during fasting, the gastric juice has invariably an acid taste and acid reaction. An examination of the gastric juice made by Professors Dunglison and Emmett[5] in 1833, confirmed Prout's statement that, on distillation, gastric juice evolves hydrochloric acid.

Lehmann's discovery of lactic acid in the gastric juice.

Prout had searched for lactic acid in the gastric juice, but had failed to find it. Lehmann, however, arrived at a different result[6], and he concluded that lactic acid is really the normal acid of the gastric juice, and that the hydrochloric acid found by Prout and others in distilling the gastric juice was produced during the process of distillation by the action of free lactic acid upon the chlorides of the juice. This view Lehmann subsequently modified, admitting the presence in the gastric juice both of "free lactic acid and lactates, in addition to free hydrochloric acid[7]."

Many observers, as Bernard and Barreswill[8], Pelouse[9] and Dundas

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[1] Prout, *Philosophical Transactions*, 1824, Part i. p. 45.

[2] Children, *Annals of Philosophy, for July*, 1824.

[3] Braconnot, *Annales de Chimie*, Vol. LIX. p. 348.

[4] Tiedemann u. Gmelin, *Op. cit.*, Vol. I. p. 151. These authors discovered the presence of hydrochloric acid in the gastric juice independently of Prout, as they inform the reader in the Preface to their great work:—'Prout gebühret die Ehre der ersten Entdeckung. Aber wir haben sie ebenfalls unabhängig von ihm, im Februar 1824, bei der Distillation verschiedener Magenflüssigkeiten entdeckt, und erst einen Monat nachher kam uns seine Abhandlung über diesen Gegenstand zu Gesicht,' (Vol. I. Preface, p. 12).

[5] Professor Dunglison's report is published in Beaumont's work, page 69.

[6] Lehmann, *Bericht d. Gesellschaft der Wissenschaften* zu Leipzig, Vol. I. 1847, p. 100.

[7] Lehmann, *Physiological Chemistry*. Cavendish Society, 1851, Vol. I. p. 93.

[8] Claude Bernard, *Leçons de Physiologie expérimentale appliquée à la Médecine*, Vol. II. (1856).

[9] Pelouse, *Comptes Rendus*, Vol. XIX. p. 1227.

Thomson[1], came to the same conclusion as Lehmann, and opinions were much divided as to the causes of the acidity of the gastric juice until the researches of C. Schmidt about to be referred to.

C. Schmidt's researches.

As a result of eighteen concordant analyses of the gastric juice of carnivorous animals, Bidder and Schmidt announced in 1852 that the gastric juice of animals which have previously fasted for a period of 18—20 hours contains *only free* hydrochloric acid and no trace of lactic acid or of any other organic acid. The gastric juice of herbivorous animals was however found to contain small but variable quantities of lactic acid, doubtless derived from the starchy constituents.

The method followed by C. Schmidt consisted in precipitating a weighted quantity (100 grammes) of gastric juice, which had been strongly acidified by nitric acid, with silver nitrate. The silver chloride thus precipitated was after suitable treatment weighed, and furnished the total amount of chlorine present either as hydrochloric acid or in combination with bases. The filtrate was then freed from silver by the addition of an excess of hydrochloric acid, evaporated to dryness, ignited, and the total amount of bases present determined.

It was found that in every case the amount of Cl present was larger than could have been the case had the whole of the bases been present as chlorides. Further, the amount of free acid present was determined by neutralizing with weighed quantities of solutions of caustic potash, as well as of lime and baryta water, and it was found that the amount of base required for neutralization corresponded to the amount of hydrochloric acid determined by the first method of research, whilst had lactic acid been present, a larger quantity of alkali would have been needed for neutralization[2].

Since these investigations of C. Schmidt it has been admitted that the cause of the acidity of the normal gastric juice is hydrochloric acid, though, as will be shewn in the sequel, Richet has of late advanced the view that the acid is conjugated with an organic body, presumably leucin.

Certain Colour Reactions depending upon the nature of the Acid of the Gastric Juice.

Various colour reactions have been discovered of late years, which enable us to discriminate between dilute solutions of mineral and of organic acids; these have been applied to the investigation of the gastric juice with the result of confirming the observations of Prout and of C. Schmidt, and of proving that the healthy gastric juice contains a free mineral acid and therefore hydrochloric acid.

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[1] Thomson, *London, Edin. and Dublin Philosoph. Mag.*, 1845.

[2] Bidder u. Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, page 44 et seq.

Rabuteau's reaction[1]. When starch mucilage is mixed with potassium iodate and iodide, a solution is obtained which is blued by a dilute solution of hydrochloric acid, but not by a dilute solution of lactic or acetic acid. The reagent employed by Rabuteau was made by adding to 50 c.c. of starch mucilage 1 grm. of potassium iodate and 0·5 grm. of potassium iodide. Rabuteau found that the gastric juice invariably caused a blueing of the solution, and this could not have been the case had the gastric juice owed its acidity to an organic acid.

Reoch's reaction. J. Reoch[2] observed that a mixture of citrate of iron and quinine and of potassium sulphocyanide was coloured red by gastric juice, just as it is by a dilute solution of mineral acids, whilst a dilute solution of organic acids does not lead to the formation of the red colour. The reagent has been modified by Szabò[3] and employed in such a manner as to permit of an estimate of the amount of mineral acid being found.

Equal volumes of half per cent. solutions of ammonium sulphocyanide and of potassio-sodic tartrate are mixed. One cubic centimetre of the pale yellow solution is coloured of a brownish-red colour by the addition of 0·5—1·0 c.c. of a dilute hydrochloric acid containing 1 part of HCl in 1000 parts, whilst a solution of lactic or of acetic acids produce no reaction unless the acid amount to 15 or 20 per cent.

With this reagent it can be shewn that the gastric juice of healthy animals contains a mineral acid.

Methyl-anilin violet reaction. A solution of methyl-anilin violet is first of all rendered blue, then green, and ultimately decolourized by dilute solutions of mineral acids, whilst dilute organic acids do not affect the violet colour. The reaction was first observed by Witz, then applied by Hilger to the detection of sulphuric acid in vinegar. Maly[4] subsequently employed it in researches connected with physiological chemistry.

It would appear, however, from the observations of Ewald[5] and of Uffelmann[6] that this reagent does not give perfectly reliable results when employed in the investigation of gastric juice as ordinarily obtained on account of the presence of proteids in it. The same remark applies to OO tropaeolin, which has been employed in the investigation of gastric juice, but which is not to be relied upon in the presence of the organic matters which it contains.

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[1] Rabuteau, *Gazette Médicale de Paris*, 1874, p. 114. This author subsequently confirmed the presence of HCl in the gastric juice by a brilliant experiment. He found that when quinine is added to the gastric juice, it is dissolved in considerable amount, and he succeeded in separating in a perfectly pure and crystallized condition hydrochlorate and not lactate of quinine. *Comptes Rendus*, LXXX. (1875), p. 61.

[2] Reoch, 'The Acidity of the Gastric Juice.' *Journal of Anat. and Phys.*, Vol. XIV. (1874), p. 274.

[3] Szabò, 'Zur Kenntniss der freien Säure des menschlichen Magensaftes.' *Zeitschrift f. phys. Chem.*, Vol. I. (1877), p. 153.

[4] Maly, 'Untersuchungen über die Mittel zur Säurebildung im Organismus,' &c. *Zeitschrift f. phys. Chemie*, Vol. I. p. 174. (See 'Qualitativer Nachweis freier Salzsäure,' &c., p. 189.)

[5] C. A. Ewald, 'Ueber das angebliche Fehlen der freien Salzsäure im Magensafte.' *Zeitschr. f. klin. Medicin*, I. 619.

[6] Uffelmann, 'Ueber die Methode der Untersuchung des Mageninhaltes auf freie Säuren.' *Archiv f. klin. Medicin*, Vol. XXVI. p. 431.

OO Tropaeolin reaction. A solution of this body (the potassium salt of phenyl amido-azobenzene-sulphonic acid) is made in alcohol. A few drops of the yellowish solution are added to the liquid to be tested. If a mineral acid be present, a fine pinkish red colour is developed, but if a dilute organic acid, no change occurs or the solution merely becomes of a more reddish yellow tint without shewing any of the pink of the HCl reaction.

This reagent may be employed in a different manner. Drops of a saturated solution are allowed to evaporate on a porcelain slab at 40° C., and, whilst at this temperature, a drop of the liquor to be tested is added, when on evaporation a violet stain is left if HCl be present. ·006 per cent. of pure hydrochloric acid can thus be detected.

Congo-Red. This colouring matter, which is soluble in water, furnishes an exceedingly delicate test for free acids, and for their detection it may be employed in aqueous solution or by saturating filter paper in it and then drying it.

Free hydrochloric acid even in very dilute solutions, changes the red to an intense blue colour, whilst organic acids cause it to assume a violet tint.

This reaction appears to the author one of the most sensitive and most useful which have been suggested for the detection and discrimination of the acids of the gastric juice.

Emerald-Green. Papers stained with this colouring matter are not affected by solutions of organic acids, however concentrated, whereas dilute solutions of hydrochloric acid change the bluish-green tint to a grass-green colour.

Phloro-glucin and Vanillin. "The reagent recommended by Günzburz contains 2 grms. of phloro-glucin and 1 gramme of vanillin dissolved in 100 c.c. of alcohol. When hydrochloric acid is added to this solution, it deposits beautiful red crystals. For the detection of the acid in gastric juice it is employed thus:—To the fluid to be tested for acid an equal quantity of the reagent is added, and the mixture evaporated in the water bath. The presence of hydrochloric acid is shewn by a delicate rose-red tint on the surface of the porcelain dish. In this way so little as 0·06 per cent. of the acid is discernible, and the reaction is not impeded by organic acid, albumin or pepton[1]."

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[1] v. Jaksch, 'Clinical Diagnosis.' Translated from the 2nd Germ. Edition by James Cagney, M.A., M.D. &c. London, Charles Griffin and Co., 1890, see pages 98 and 99.

Benzo-Purpurin. A still more sensitive colour test is said to be furnished by benzo-purpurin, although the author cannot speak of it from his own experience. The following account is taken from v. Jaksch.

"Five milligrammes will serve to shew 0·39 milligrammes of acid dissolved in 6 c.c. of water (Hellström), causing the dark-red colour of the solution to give place to a light violet. A similar change is effected with acetic, formic, and lactic acids; but the colour obtained with organic acids is rather a brownish-violet, and requires a greater quantity of the latter for its production; in the case of acetic acid, not less than 0·84 milligramme. Test papers may be prepared by soaking strips of filter paper in a saturated watery solution of benzo-purpurin, and subsequently allowing them to dry. If one of these be placed in gastric juice, it will immediately stain a dark blue, provided hydrochloric acid be present in a proportion not less than 0·4 grm. to 100 c.c. A brownish-black tint may be due to the presence of organic (lactic or butyric) acids; or from a mixture of these with hydrochloric acid. The ambiguity in this case may be dispelled by placing the paper so stained in a test tube and shaking it up with sulphuric ether, when so much of the colour as is due to the presence of organic acid will speedily disappear, leaving a lighter stain, or restoring the paper to its original tint. If hydrochloric acid alone be present no change will be effected in this way, and even after the lapse of twenty-four hours the blue stain will be only slightly displaced[1].

Is the Acid of the Gastric Juice free Hydrochloric Acid?

The observations of Carl Schmidt proved that the gastric juice contains a chlorine-containing acid uncombined with bases, and taken in connection with the facts, firstly that the gastric juice yields free hydrochloric acid on evaporation, secondly, that the gastric juice yields the same reactions with certain reagents (methyl-violet, Reoch's reagent, OO tropaeolin) as a dilute solution of a mineral acid, it would appear in the highest degree probable that the chlorine-containing acid is in reality hydrochloric acid.

It has however been maintained that in some particulars the gastric juice does not behave exactly like a dilute solution of hydrochloric acid of the same degree of acidity. Some of the supposed points of difference depend however upon errors of observation, and others are explained by the modifying influence exercised upon the hydrochloric acid by the organic matters of the gastric juice.

It was asserted by Blondlot that gastric juice does not decompose calcium carbonate, and the supposed fact was used as an argument in support of Blondlot's theory that the acidity of the gastric juice was due to acid sodium phosphate. "Dumas, Melsens and Bernard have found that not only the carbonate, but also basic calcium phosphate is soluble in gastric juice, as also are even lead, zinc and iron, hydrogen being simultaneously developed[1]."

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[1] v. Jaksch, *Op. cit.*, p. 99.

Bernard and Barreswill[2] also maintained that the insolubility of calcium oxalate in the gastric juice proved that it did not owe its acidity to hydrochloric acid, inasmuch as a solution of hydrochloric acid containing one part of the acid per mille dissolves the salt. It has been shewn, however, that the organic matters of the gastric juice must be the hindering cause, as when gastric juice is neutralized, and then its natural acidity restored by the addition of hydrochloric acid, the solution is incapable of dissolving C_2O_4Ca.[3]

Laborde[4] thought he had discovered an important point of difference between gastric juice and a dilute solution of hydrochloric acid of corresponding acidity, by shewing, first, that such a dilute solution of pure hydrochloric acid when treated with a solution of cane-sugar, possesses a more powerful inverting action than the gastric juice; the inverting power of the latter corresponding to that of a solution of lactic acid; secondly, that when starch is heated with a dilute solution of hydrochloric acid (containing only 0·25 of HCl per mille) at 155° for two hours, it is entirely converted into dextrin and grape-sugar, whilst with gastric juice under the same circumstances the conversion of starch is much less complete.

Szabò[5] has subjected Laborde's facts to a searching investigation, with the result of shewing that peptones interfere with the action exerted by dilute hydrochloric acid upon starch; in spite of this interference, gastric juice has an action which is much more intense than that of dilute lactic acid, and which is essentially the same as that exerted by dilute hydrochloric acid.

The Researches of Richet.

An important series of researches has been performed by Richet of late, which in the main confirm the researches of Schmidt, though they have led the author to an hypothesis which is yet unproved.

Richet, following in essential particulars Schmidt's method of analysis, came to the conclusion in the first place that the gastric juice contains a free chlorine-containing acid.

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[1] Lehmann, *Physiological Chemistry*. Cavendish Society, edition 1853, Vol. II. p. 44.

[2] Bernard and Barreswill, Claude Bernard, *Leçons de Phys. Expér. appliquée à la Médecine*, 1856, Vol. II.

[3] Kühne, *Lehrbuch d. physiol. Chemie*, p. 31.

[4] Laborde, 'Nouvelles recherches sur l'acide libre du suc gastrique.' *Gazette médicale de Paris*, 1874, No. 33—34.

[5] Szabò, 'Beiträge zur Kenntniss der freien Säure des menschlichen Magensaftes.' *Zeitsch. f. phys. Chemie*, Vol. I., p. 140.

By adopting a new method, to be immediately referred to, he proved also more satisfactorily than had ever been done before, that fresh gastric juice owes its acidity to a single acid, though after the gastric juice has been kept for some time it undergoes decompositions which lead to the appearance of more than one acid. Richet has however been led to the conclusion that the chlorine-containing acid of the gastric juice is not free hydrochloric acid, but an acid conjugated with leucine.

Berthelot's method of determining the nature of acids in solution by their 'coefficient of distribution.'

In endeavouring to decide the question whether the gastric juice contains a mixture of acids or one acid only, Richet[1] made use of a method devised by Berthelot, which will doubtless be of much use in future researches in physiological chemistry.

The method rests upon the following principles: When a watery solution of an acid is shaken up with an equal volume of pure ether, the latter fluid takes up a certain proportion of the acid, which varies with the nature of the acid and with the temperature. The ratio of the amount of acid contained in the ether to that remaining in the water is therefore constant for each acid at a given temperature; by dividing the number expressing the acidity of the water (expressed for instance in terms of a standard alkaline solution used) by the number expressing the acidity of the ether, we obtain as a quotient the "*coefficient de partage*" of Berthelot, which we may term the "*coefficient of repartition*" or, perhaps better, '*coefficient of distribution.*' In the case of mineral acids, the amounts dissolved by the ether are very small, and the coefficients are represented by high numbers; in the case of the organic acids, the amount soluble in ether is large, and the coefficients are small numbers.

The following are the "coefficients of repartition" of some organic acids:

Lactic acid....	C = 8·8—11·0
Succinic „	C = 6·0
Benzoic „	C = 9·5
Acetic „	C = 1·4
Tartaric „	C = 96·0

When two or more acids are, however, present in a watery solution, the determination of the coefficient of each is also a possible, though necessarily a more complex, operation.

By employing this method, Richet found that perfectly fresh gastric juice contains essentially one acid with a very high coefficient of distribution—an acid, that is, which, as the mineral acids, is almost insoluble in ether.

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[1] Ch. Richet, *Le Suc Gastrique chez l'homme et les Animaux, ses propriétés Chimiques et Physiologiques.* Paris, 1878.

How valuable the method is in establishing that lactic acid could not be the cause of the acidity of the gastric juice is shewn by the following experiment:

Pure gastric juice was shaken with ether and the acidity of the acid and ether afterwards determined. The coefficient of repartition was found to be 137·1, i.e. the amount of acid held in solution by the water was 137·1 times greater than that held in solution by an equal volume of ether. A certain quantity of the same gastric juice was now treated with solution of barium lactate. By the action of the HCl of the juice upon this salt there would obviously be set free an equivalent quantity of lactic acid, which should now be *the* free acid of the juice. The coefficient of repartition was now determined and found to be 9·9, i.e. that of lactic acid.

When the gastric juice is kept, however, as well as during the process of digestion, there are formed other acids, such as lactic, butyric, and acetic, the occurrence of which will be again referred to, in discussing the changes which go on in the stomach during digestion.

Although Richet concludes from his researches that the gastric juice, when fresh, contains but one acid, and that a chlorine-containing mineral acid, he is led by an experiment now to be referred to, to the opinion that this is not free hydrochloric acid.

Hydrochloric acid is so slightly soluble in ether that it does not possess an appreciable coefficient of repartition. If however, as M. Berthelot shewed, an alkaline acetate is added to dilute hydrochloric acid, acetic acid is set free and a chloride formed; if then we shake the mixture with ether we obtain a number which is essentially the coefficient of repartition of acetic acid. On trying this experiment with gastric juice, Richet did not however obtain a coefficient low enough for acetic acid; it was from 5 to 5·8 instead of 1·7. This fact Richet explains by saying that the quantity of acetic acid set free by the gastric juice was to that which hydrochloric acid would have set free as 0·4 to 1, and from this he concludes that the acid of the gastric juice cannot be free hydrochloric acid.

Richet further has found that by digesting at 45° dilute hydrochloric acid with the mucous membrane of the fourth stomach of a calf, a solution is obtained which behaves in respect to acetate of soda exactly like gastric juice. Upon grounds which appear to the author very slender, and the chief of which is that he has, as Kühne has done long ago, succeeded in separating traces of leucine from the mucous membrane of the stomach, Richet believes that there is formed in this case, a conjugate acid of leucine and hydrochloric acid, and that such a conjugate acid is the normal acid of the gastric juice. The author in conjunction with Dr Haslam has prepared hydrochlorate of leucine and finds that the salt does not in relation to pepsin act as an acid, i.e. that when pepsin is mixed with a watery solution of the compound of hydrochloric acid and leucine, and the mixture is heated to 40° C., it possesses no digestive properties. These experiments appear to negative conclusively the hypothesis that the hydrochloric acid of the gastric juice exists in combination with leucine.

The progress of research will probably shew that the deviation from strict normality exhibited by the hydrochloric acid of the gastric juice is due to the organic matters which it contains. Nevertheless the information obtained by Richet on the main question—viz. that the acid of the fresh gastric juice is essentially one, and that it behaves to ether as a mineral acid, must be considered as having added very greatly to our knowledge of the gastric juice, and taken in connexion with other facts also discovered by him, to our knowledge of gastric digestion.

Lactic and Butyric Acids in the Gastric Juice.

Although the evidence afforded by various colouring matters supports that furnished by other methods of research and leads us to conclude that the essential acid of the gastric juice is hydrochloric acid, there can yet be no doubt that the juice obtained during the processes of digestion contains other acids and especially lactic, butyric and acetic acids, together frequently with free vegetable acids such as malic, the result of the decomposition of salts contained in the food. Of these acids lactic acid is probably a constant physiological constituent of the juice.

Method of determining presence of lactic acid in gastric juice.

In order to test for lactic acid in gastric juice, the fluid should be repeatedly shaken with ether, and the ethereal solutions allowed to evaporate spontaneously, the residue being dissolved in water and subjected to the following tests:

1. A dilute solution of ferric chloride is made by adding from 2 to 5 drops of a 10 per cent. solution to 50 c.c. of water. Such a dilute solution is almost colourless, possessing only, when examined in thin layers, a very faint straw colour. When a trace of free lactic acid is added to it however, the colour is at once changed to a much deeper yellow-straw colour, a result which is not produced by either hydrochloric, acetic or butyric acids.

2. Dilute a 4 per cent. solution of carbolic acid with twice its volume of distilled water and add to it a few drops of a solution of ferric chloride, which will give rise to a violet colour. When a solution containing a trace of lactic acid is added to a small quantity of this violet coloured solution, the colour disappears or rather the violet is changed to a yellow colour (Uffelmann's Reaction[1]). This reaction is inferior in value to the first mentioned.

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[1] Uffelmann, *Deutsches Archiv für klin. Med.* Vol. XXIV. (1884), p. 437.

Methods of separating butyric and other volatile acids.

The gastric juice is distilled, and if butyric and acetic acids are present they will pass in the distillate where they may be detected by their physical characters or by preparing from them barium or lime salts, and determining the proportion of barium or calcium which they contain. The determination of the 'coefficient de partage' of the distillate affords moreover an easy and good method of identification.

SECT. 9. SEATS OF FORMATION OF THE MUCUS, PEPSIN, AND HYDROCHLORIC ACID IN THE STOMACH. THE ANTECEDENTS OF THESE BODIES.

SECRETION OF MUCUS.

During fasting, the mucous membrane of the stomach is always covered by a thin layer of mucus, which is doubtless being continuously secreted, though, as was stated previously, the quantity formed in the normal state of the stomach is less than would be surmised from an examination of the stomach of dogs with gastric fistulae, in which the cannula keeps up a constant condition of irritation. When gastric digestion is proceeding, the secretion of mucus occurs even more actively than before. The gastric mucus is produced by the cylindrical epithelium cells, which covers the whole internal surface of the stomach, and of which the protoplasm undergoes a transformation into mucin[1].

THE PYLORIC GLANDS AND THE PYLORIC JUICE.

It has already been remarked that the gastric glands are divisible into two classes; of which the one, the so-called mucous glands of the older authors, occur in some animals alone at the pyloric end of the stomach, the other so-called peptic glands being situated at the cardiac end of the organ.

As is implied by the name mucous glands, it was until lately held that the pyloric glands are mucus-secreting glands, and that the elements of the gastric juice were elaborated in the so-called peptic glands.

Since the time of Eberle it has been known that any part of the mucous membrane of the stomach will, if digested with dilute acids, furnish an active digestive fluid, yet it was soon recognized (Wasmann) that the digestive fluid prepared with the mucous membrane of the cardiac end of the stomach is much more active than that prepared with the pyloric end.

When v. Wittich's method of extracting pepsin from the gastric mucous membrane by means of glycerin was adopted it was found too that whilst glycerin extracts, whether of pyloric or cardiac end, contained pepsin, in the latter case the extract was very much more active than in the former, and much freer from mucin. The view was held by many that the pyloric glands had nothing to do with the secretion of the essential constituents of the gastric juice, and that the pepsin which can be extracted by glycerin or by dilute hydrochloric acid was pepsin which had been merely absorbed by the mucous membrane of the pyloric region, though elaborated by the glands at the cardiac end. In the discussion which took place on this interesting question, v. Wittich[1] took a leading part in support of the *imbibition-theory* of the origin of the pepsin of the pyloric region, whilst Ebstein and Grützner undertook to prove that pepsin is not merely a product of the activity of the peptic glands proper, but likewise of the pyloric glands[2].

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[1] Heidenhain, Hermann's *Handbuch*, Vol. v. Part i. Chap. iii. p. 122.

Pepsinogen.

Ebstein and Grützner's theory of a pepsinogenic substance.

The views of Ebstein and Grützner were largely founded upon the observation made by them that a hydrochloric acid extract of a gastric mucous membrane digested albumen very much more actively than a glycerin extract of a gastric mucous membrane—the two extracts being diluted with hydrochloric acid to an equal extent before being tested. The difference in the digestive power of the two extracts held both in the case of the fundus and of the pyloric region of the stomach. The method of comparing digestive power was that of Grünhagen. On the assumption that glycerin takes out all the pepsin from the gland-cells, it followed that there was some substance in the mucous membrane which in some way or other under the action of hydrochloric acid gave rise to pepsin. This pepsinogenic substance, as they called it, they considered might be a combination of pepsin with the proteids of the gland-cells, or not completely formed pepsin. The proof however is not a valid one, since glycerin does not extract all the pepsin from proteids; thus, as noticed by Wittich and by Ebstein and Grützner themselves, if fibrin be placed in a glycerin extract of pepsin, it takes up a portion of pepsin, and this cannot be extracted from it by glycerin; and the same holds if a neutralized acid extract be taken instead of a glycerin extract. Ebstein and Grützner came to the conclusion that the pepsinogenic substance was not extracted by glycerin: that it was split up by dilute sodium chloride solution, as well as by hydrochloric acid, to give rise to pepsin. On both of these points compare below.

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[1] v. Wittich, 'Ueber die Pepsinwirkung der Pylorusdrüsen.' Pflüger's *Archiv*, Vol. VII. p. 18.

[2] W. Ebstein und P. Grützner, 'Ueber den Ort der Pepsinbildung im Magen.' Pflüger's *Archiv*, Vol. VI. p. 1.

Schiff's propepsin.

Schiff observed that when a stomach was treated with acidulated water, the digestive power of the fluid increased for some weeks. This he considered to be due to the presence of 'propepsin,' which was only slowly converted into pepsin. Apparently however pepsinogen is converted into pepsin with great rapidity by even very dilute hydrochloric acid.

Langley's experiments establishing the existence of pepsinogen.

Although Ebstein and Grützner had brought forward evidence which rendered the existence of *pepsinogen* most probable, the most conclusive proof of its reality has been adduced by Langley. This observer shewed that pepsin and its precursor pepsinogen behave very differently when digested at 40° with solution of sodium carbonate, containing from 0·5 to 1·0 per cent. of Na_2CO_3, the former body being very readily destroyed by it, the latter comparatively slowly; the action of the alkaline salt causing first, however, the appearance by degrees of pepsin. When an active hydrochloric acid extract of the mucous membrane of the stomach is neutralised and digested with the above solution and afterwards re-acidified, it is found to have lost its proteolytic power. On the other hand, when a watery extract of the mucous membrane is digested for an equal time with solution of Na_2CO_3 of the same strength, on subsequently acidifying it is found to possess proteolytic powers[1].

Pepsinogen is, according to Langley, soluble in water, and so is soluble in glycerin, unless this be anhydrous; it is, however, more soluble in salt solution.

From his researches Langley concludes, "That the gastric glands contain no ferment during life, but much zymogen or substance capable of giving rise to ferment."

"That by far the greater part of the zymogen can be seen in the chief (central) cells in the form of granules."

"That during digestion, the granules are usually used up in such a manner as to give rise to an outer non-granular and an inner granular zone in the chief cells."

Further researches of Langley in association with Edkins.

Continuing his researches in conjunction with Edkins[2], Langley has been able to confirm his original conclusions and to add considerably to our knowledge of the relations of pepsinogen and pepsin and the circumstances under which the latter is produced from the former. The following were their chief results:

Pepsin is very rapidly destroyed by alkalies and by alkaline salts. The principal conditions which influence the rate of destruction of pepsin by sodium carbonate are, the strength of the solution of the alkaline salt, the time during which it is allowed to act, the temperature of the mixture, and the amount of proteids present. The mere act of neutralising an acid pepsin solution may destroy a considerable part of the pepsin. When equal volumes of a fluid containing pepsin or 1 per cent. solution of sodium carbonate are well mixed, the greater part of the pepsin is destroyed in fifteen seconds; in a neutralised acid extract of the gastric mucous membrane of a cat the amount thus destroyed may be nearly 97 per cent. of the whole. Even very dilute sodium carbonate (0·005 per cent.) will cause an appreciable destruction of pepsin in one or two hours at the body temperature, provided proteids are present in small amount only.

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[1] J. N. Langley, 'On the Histology of the Mammalian Gastric Glands, and the relation of Pepsin to the Granules of the chief Cells.' *Journal of Physiology*, Vol. III. p. 269.

[2] J. N. Langley and J. S. Edkins, 'Pepsinogen and Pepsin.' *Journal of Physiology*, Vol. VII. p. 371.

Proteids lessen the rate of destruction of pepsin, probably by combining with the alkali or alkaline salt.

Pepsin prepared from a frog is less rapidly destroyed than pepsin prepared from a mammal.

The difference between pepsinogen and pepsin on their behaviour to reagents is one of degree only, and not one of kind. Pepsinogen, like pepsin, is destroyed by alkalies and alkaline salts, but the destruction is much slower. Pepsinogen is very rapidly converted into pepsin by dilute mineral acids; at 20° C. all or nearly all the pepsinogen present in an aqueous extract of a cat's gastric mucous membrane, may be converted into pepsin in 60 seconds by 0·1 per cent. of HCl. In the absence of acid, pepsinogen is fairly stable; in neutral and in alkaline solutions its conversion is slow, and in a glycerin extract it may remain unchanged for years. Pepsinogen is not affected by a stream of oxygen passed through it.

Since the aqueous extract of the gastric mucous membrane of a hungry animal does not lose peptic power, or loses very little, on brief treatment with sodium carbonate, it follows that pepsinogen, but little or no pepsin, is present in the gastric glands during abstinence.

In consequence of the rapidity of conversion of pepsinogen into pepsin, it is difficult to be certain whether pepsin is or is not present in the gastric glands during digestion and after the injection of peptone into the blood. In both cases, acid gastric juice is present in the stomach and it is probable, since the glands are secreting at the moment of death, that a little acid remains in the lumina of the glands and, before it can be neutralised, soaks into the gland-cells and changes some pepsinogen to pepsin. In fact, pepsin is sometimes present in an extract prepared from the gastric glands of a digesting animal, but it is not always so.

Carbonic acid when passed through an aqueous extract of a frog's œsophageal glands for about an hour destroys nearly the whole of the pepsinogen. Certain salts increase the rate of destruction, whilst peptone greatly delays the action, and albumin and globulin likewise do so, though to a much less extent. Carbonic acid destroys pepsin also, but less readily than pepsinogen.

Both pepsinogen and pepsin are rapidly destroyed when heated to 55° C—57° C.

The Experiments of Klemensiewicz and Heidenhain on the Pyloric Secretion.

Prompted by the very wonderful experimental procedure by which Thiry had thrown light upon the intestinal secretion, Klemensiewicz conceived the idea of exposing the stomach of a living animal and making two parallel incisions right through the pyloric part of the organ so as to obtain a cylinder lined internally by pyloric mucous membrane and retaining its essential vascular and nervous connexions. The cylinder had one of its ends closed up by sutures and thus a tube was made which was twisted round; the edges of the open mouth of the tube were then stitched to the edges of the incision which had been made in the abdominal wall. Thus was obtained a tube whose walls were constituted by pyloric mucous membrane and which opened on the external surface of the body. The two portions of the stomach from which the above-mentioned intermediate portion had been removed were now brought into contact and united by sutures, so as to re-establish the continuity of the organ[1]. All the dogs experimented upon by Klemensiewicz ultimately succumbed to the formidable operation just described, yet the experimenter was able to ascertain that the pyloric tube, which he had established, secreted an alkaline, viscous, liquid (succus pyloricus) which by itself did not digest proteids, but which did so after acidulation with hydrochloric acid.

Interesting, and especially suggestive, as were the experiments of Klemensiewicz, they could not be held absolutely to disprove the *imbibition-theory* of the pyloric pepsin, for, as the animals survived but for a short time, the pyloric juice might be supposed to contain some pepsin which had been elaborated, before the operation, in the peptic glands of the fundus, and subsequently imbibed by the pylorus.

Heidenhain[2], however, repeated Klemensiewicz's procedure. The adoption of Lister's antiseptic method of wound treatment enabled this skilled experimenter to succeed, in three operations out of six, in establishing a permanent independent pyloric tube which enabled the secretion to be observed, in one case, for a period of five months.

From these observations it results that after food has entered the stomach, there is slowly set up a secretion of pyloric juice, which is at its height about the fifth hour. The secretion, which is scanty, always has an alkaline reaction, is viscid and is rich in pepsin and in rennet-ferment. When acidulated with HCl it digests fibrin abundantly. It contains no diastatic ferment.

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[1] Klemensiewicz (Graz), 'Ueber den Succus Pyloricus.' *Sitzungsber. d. k. Acad. d. Wiss.* Wien, Vol. LXXI. 1875. March 18.

[2] Heidenhain, 'Ueber die Pepsinbildung in den Pylorusdrüsen.' Pflüger's *Archiv*, Vol. XVIII. (1878), p. 169. A description of the method of carrying out the operation illustrated with a diagram shewing the direction of the various incisions of the stomach is given by Heidenhain in his article entitled 'Physiologie der Absonderungsvorgänge. 2 Abschn: Der Magen,' in Hermann's *Handbuch*, Vol. v. Pt. i. p. 110.

These experiments have conclusively proved that the glands of the pyloric region of the stomach take some part in the formation of pepsin, though of the total amount secreted by fundus and pylorus, the latter is unimportant compared with the former.

THE GLANDS OF THE FUNDUS. THE CELLS WHICH PRODUCE PEPSIN AND THE CELLS WHICH PRODUCE ACID.

By a procedure similar to that which he employed in establishing in a living animal a tube composed of the pyloric portion of the stomach, Heidenhain succeeded in separating from its continuity with the rest of the stomach a part of the fundus and stitching it into the form of a tube, of which he connected the opening with the external abdominal wall. In one case an animal which had been subjected to this operation lived for 33 days and allowed Heidenhain to collect the pure secretion of the fundus.

A few minutes after introduction of food into the stomach, secretion commenced in the sac and continued throughout the whole period of digestion. The fluid secreted was watery, of acid reaction, and contained pepsin; it had, in short, all the characters of gastric juice.

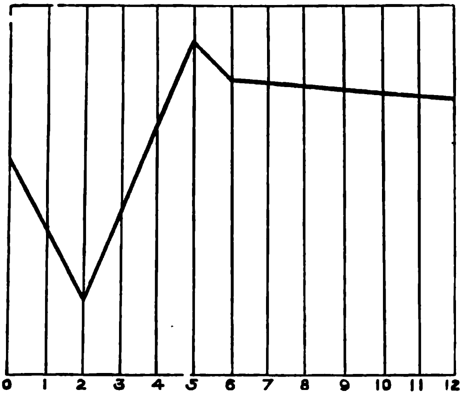

FIG. 8. CURVE EXHIBITING THE AMOUNT OF PEPSIN IN A GIVEN AMOUNT OF THE SECRETION OF THE FUNDUS, DURING SUCCESSIVE HOURS OF THE DIGESTIVE PROCESS (HEIDENHAIN).

Whilst the pyloric glands thus secrete an alkaline liquid containing pepsin, the glands of the fundus secrete both pepsin and acid. The question then suggests itself, Where is the seat of the formation of pepsin, and where that of the formation of acid? It has already been said that a considerable histological resemblance exists between the pyloric epithelial cells and the central cells of the fundus, the one form passing by transitions into the other; and a large number of facts indicate that the resemblance in structure is accompanied by an essential identity in the processes which have their seat in them.

HISTOLOGICAL CHARACTERS OF THE GASTRIC GLANDS DURING FASTING AND DIGESTION, AND RELATION OF THESE TO THE AMOUNT OF PEPSIN PRESENT.

Our knowledge of the changes which the secretory epithelium cells of the gastric glands undergo during digestion, or rather the appearances which they present during abstinence from food and at various stages of the digestive process, is derived from the researches of Grützner and Heidenhain who have studied the glands after they have been hardened in alcohol, and from those of Langley and Sewall who have traced the changes in the living glands.

Taking the case of the glands hardened in alcohol first, it is found that the central cells of the cardiac glands and the deeper cells of the pyloric glands, after long abstinence from food are somewhat swollen, pale, finely granular, and do not stain readily. During the early stages of digestion the cells appear somewhat larger, more granular and more easily stained; whilst at the close of the digestive process the cells are much diminished in size, as it were shrunken, and are stained much more deeply than during the early stages.

From Langley and Sewall's observations of the fresh and living glands, unacted upon by reagents, we know that after long abstinence the cells are studded with granules, which diminish during the act of secretion, and collect near the lumen, the outer zone of the cell appearing clear. The granules are most abundant in those regions of the stomach in which the amount of pepsin is found to be largest. From these facts it is concluded that "the conspicuous granules in the chief cells are directly connected with the formation of ferment[1]." According to this view, which has also been adopted by Nussbaum, the changes in the gastric glands closely resemble the changes which occur in the pancreas.

Grützner's curves representing the fluctuations in the amount of pepsin in the mucous membrane of the fundus and pylorus.

Grützner, and after him Langley and Sewall, have endeavoured to ascertain the relative amounts of pepsin present in the stomach at varying times, and the former observer has expressed his results in the form of the curves which are appended (p. 108), and which exhibit the variations in the amount of pepsin present in the mucous membrane of the fundus and of the pylorus during abstinence and in successive hours following digestion. The equal numbered intervals in the abscissa-line represent hours; the relative amounts of pepsin are represented by the ordinates. These curves exhibit in a startling manner the remarkable want of coincidence in the richness in pepsin of the mucous membrane of the fundus and pylorus. In the experiments which furnished the data for these curves, the mucous membrane of the pylorus, and of the fundus, respectively, were extracted first with glycerin and afterwards with hydrochloric acid. The curves indicate that the relationship between the amount of pepsin in the pyloric mucous membrane and that of the fundus is a very varying one.

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[1] J. N. Langley and H. Sewall, 'On the Changes in Pepsin-forming Glands during Secretion.' *Journal of Physiology*, Vol. II. pp. 81 et seq.

THE SEAT OF THE FORMATION OF THE ACID OF THE GASTRIC JUICE.

Those facts have already been adduced which are considered to prove that the pepsin of the gastric juice is formed in or by the central cells of the glands of the fundus of the stomach and by the pyloric glands, and that the border cells of the former glands form the acid. Some of the most important evidence which appears to shew that these border cells are the active acid-forming structures has already been referred to, particularly that from that part of the stomach of which the glands are free from border cells (pyloric region) can be obtained a juice rich in mucus and containing pepsin, but alkaline.

Evidence in the same direction is afforded by a study of the œsophagus and stomach of the frog. In this animal the principal seat of the production of pepsin is in the œsophagus, where glands are found of which the secreting cells are somewhat like the chief cells of the gastric glands; but the total amount of pepsin in the stomach is not much less than in the œsophagus. These glands secrete an alkaline fluid. On the other hand, the glands of the stomach consist—apart from mucous cells—of cells somewhat like border cells, and here an acid juice is formed[1].

The view was formerly held that the border cells were not acid-producing but pepsin-forming cells, and it formerly received the support of Nussbaum[2] and of Edinger[3], but on grounds which warranted no such conclusion. Nussbaum in 1881 however adopted the opinion that the chief cells are the principal sources of pepsin. The first of these writers observed that

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[1] H. v. Swiecicki, 'Untersuchungen über die Bildung und Ausscheidung des Pepsins bei den Batrachiern.' Pflüger's *Archiv*, Vol. XIII. (1876), p. 444. Langley and Sewall, 'On the Changes in Pepsin-forming Glands during Secretion.' *Journal of Physiology*, Vol. II. (1879—80), p. 281. Langley, *Phil. Trans.* 1881.

[2] Nussbaum, 'Die Fermentbildung in den Drüsen.' Habilitationsschrift. Bonn, 1876 (not seen).

[3] Edinger, 'Zur Kenntniss der Drüsenzellen des Magens besonders beim Menschen. *Archiv f. mikroscop. Anat.*, Vol. XVII. p. 194—212.

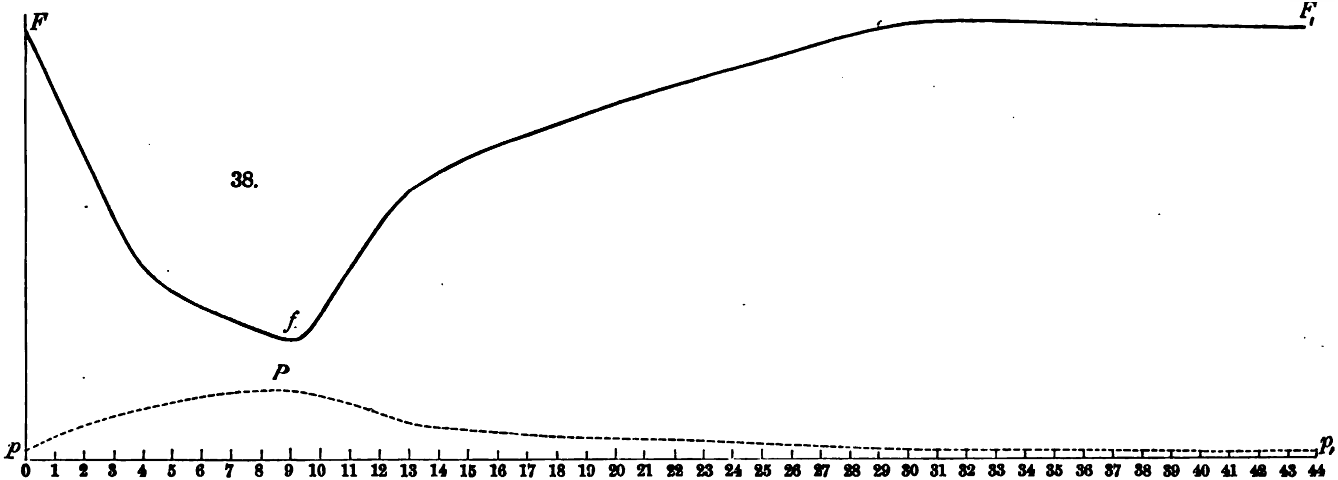

FIG. 9. CURVES EXHIBITING THE AMOUNT OF PEPSIN IN THE MUCOUS MEMBRANE OF THE FUNDUS *F*, AND OF THE PYLORUS *P*, DURING THE COURSE OF DIGESTION. THE NUMBERS ON THE ABSCISSA-LINE INDICATE HOURS SINCE THE LAST MEAL. (GRÜTZNER.)

the border cells of the stomach were coloured of a dark colour by perosmic acid, whilst the chief cells were not, and explained the reaction by supposing that the coloured particles consist of ferment, because solutions of enzymes are similarly darkened. The views of Nussbaum on this matter may be set down as undeserving of serious discussion.

Although we may then hold it as proved that the formation of acid in the gastric juice is associated with the border cells, direct observations would appear to shew that in the deeper part of the gastric glands the reaction is never acid, and that it is so only on the surface of the stomach and near the mouths of the glands.

Claude Bernard's experiment on the reaction of the mucous membrane of the stomach.

If a solution of lactate of iron and ferrocyanide of potassium be mixed, no blue colour is produced except when a free acid be added. As these salts do not exert any poisonous action, it occurred to Claude Bernard[1] to inject their solutions into the blood and to notice what structures in the stomach, if any, would assume a blue colour. It was found that the surface of the stomach was stained of a Prussian-blue colour, but that the deeper portions of the mucous membrane were unstained.

Similarly it has been found that whilst the surface of the mucous membrane has an acid reaction, on making sections parallel to the surface, so as to cut the glands at some distance from their mouths, the exposed surface is not acid. This experiment is unworthy of serious discussion, seeing that lymph, blood, and the cells themselves would all tend to neutralise the extremely small amount of acid present in the glandular lumina.

Observations have likewise been made with a view to determine whether the border cells have an acid reaction, but with entirely negative results[2].

Are we from these observations to conclude that whilst the border cells of the glands of the fundus are essential to the production of the free acid of the gastric juice, they are not the actual seats of its formation, and that it is only on the free surface of the mucous membrane of the stomach that acid is liberated? Even Claude Bernard hesitated to draw this inference from his experiment. It is not only conceivable, but probable, that, so soon as formed, the acid secretion of the gastric glands is poured into the stomach, so that no appreciable quantity of acid is retained in the gland, able to give an obvious colour reaction in the deeper part of the mucous membrane. This explanation does not account for the undoubted failure to prove that the border cells have an acid reaction; but the absence of an acid reaction does not militate against the theory that they are in reality the acid-forming cells of the stomach. As has been justly remarked[1], a secreting cell does not necessarily contain the products which are characteristic of its activity, and which it contributes to the secretion, and which appear in some cases to be at once removed from their place of origin: thus the hepatic cells contain no bile-colouring matter, and no bile acids; though unquestionably these bodies are formed first in those secreting cells.

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[1] Claude Bernard, *Leçons sur les propriétés physiologiques et les altérations pathologiques des liquides de l'organisme.* Paris, 1859, Vol. II. p. 375.

[2] Lépine, 'Recherches expérimentales sur la question de savoir si certaines cellules des glandes (dites à pepsine) de l'estomac présentent une réaction acide.' *Gazette médic. de Paris*, 1873, p. 689. Heidenhain, see 'Die Bildung der Säure des Magensaftes.' Hermann's *Handbuch*, Vol. v. Part i. p. 150 (small type).

To summarize. All the evidence which we possess points to the border cells which are found in certain of the gastric glands, as the seats of the formation of the free acid of the gastric juice; and the value of this evidence is not diminished by the fact that the cells which are supposed to possess this power possess no acid reaction, inasmuch as other undoubted cases are known in which the products of secretion cannot be discovered in the gland cells which form them.

THEORIES AS TO THE MODE OF PRODUCTION OF THE ACID OF THE GASTRIC JUICE.

Various attempts have been made to explain the nature of the chemical operations which may lead to the separation of hydrochloric acid by the gastric glands.

That the acid is derived from the decomposition of chlorides may be assumed, and the assumption is confirmed by the observation of Grützner, that coincidently with the greatest richness in pepsin of the gastric mucous membrane it is likewise richest in chlorides[2].

It was further observed in the first instance by Bence Jones, and afterwards confirmed by Roberts and by Maly, that during active digestion when the gastric juice is being abundantly poured out there is a diminution in the acidity of the urine, which may become neutral or alkaline.

Brücke's hypothesis.

Brücke[3] surmised that under the influence of their secretory nerves the gastric glands possessed the power of decomposing chlorides electrolytically (?), and of directing the hydrochloric acid to the stomach, whilst the bases, accumulating in the blood, were excreted by other channels.

Ralfe's experiments.

Dr Ralfe[4] gave some support to the electrolytic hypothesis by shewing that when a weak current of electricity is passed through a U-tube, in one limb of which is a solution of sodium bicarbonate, and in the other a solution of sodium chloride, a membranous diaphragm separating the two solutions, the liquid at the positive pole acquires an acid reaction, owing to the presence of free hydrochloric acid, whilst that at the negative becomes more alkaline. The reaction which takes place occurs according to the following equation.

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[1] Heidenhain, *Op. cit.*, p. 150.

[2] Grützner, *Neue Untersuchungen über die Bildung und Ausscheidung des Pepsin.* Breslau, 1875, p. 52.

[3] Brücke, *Sitzungsber. d. Wiener Akad.*, Vol. xxxvii. (1859), p. 131, also *Vorlesungen über Physiologie*, Wien, 1875, p. 299.

[4] Ralfe, *Lancet*, 1874, 2, 29.

$$NaHCO_3 + NaCl = Na_2CO_3 + HCl.$$

To both Brücke's and Ralfe's views it must be objected that they are purely speculative, and that they postulate the agency of forces, to bring about the decomposition, which cannot be proved to be in operation.

Maly's first investigations.

Maly ascertained that when an alkaline chloride is mixed with lactic acid and the mixture is subjected to dialysis, free hydrochloric acid diffuses, the four bodies indicated in the subjoined equation resulting from the interaction of sodium chloride and lactic acid.

$$\underbrace{2\,NaCl}_{\substack{\text{Sodium}\\ \text{chloride}}} + \underbrace{2\,C_3H_6O_3}_{\substack{\text{Lactic}\\ \text{acid}}} = \underbrace{NaCl}_{\substack{\text{Sodium}\\ \text{chloride}}} + \underbrace{NaC_3H_5O_3}_{\substack{\text{Sodium}\\ \text{lactate}}} + \underbrace{C_3H_6O_3}_{\substack{\text{Lactic}\\ \text{acid}}} + \underbrace{HCl}_{\substack{\text{Hydrochloric}\\ \text{acid}}}$$

Assuming that lactic acid were first of all produced in the gastric mucous membrane, the subsequent liberation of hydrochloric acid would thus be easily explained. Maly found, however, no evidence of such a formation of lactic acid, and therefore concluded that the free hydrochloric acid of the gastric juice was due to a dissociation of the chlorides, without the interaction of any acid[1].

Maly's second investigation and more recent theory.

The blood, Maly remarked, is a liquid possessed of an alkaline reaction, which, however it derives from the presence of two acid salts, sodium bicarbonate ($NaHCO_3$) and disodic phosphate (Na_2HPO_4). But the blood contains an excess of carbonic acid. When this body acts upon alkaline disodium hydrogen phosphate Na_2HPO_4, it gives rise to NaH_2PO_4 and $NaHCO_3$, as shewn in the following equation:—

$$Na_2HPO_4 + CO_2 + H_2O = NaHCO_3 + NaH_2PO_4.$$

Acid sodium phosphate is a body possessed of a decidedly acid reaction, which however is concealed when its solution is mixed with an excess of the alkaline phosphate. Now when acid sodium phosphate coexists in solution with sodium chloride, free hydrochloric acid is set free, as shewn in the following equation:—

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[1] Maly, 'Untersuchungen über die Quelle der Magensaftsäure.' *Annalen d. Chemie u. Pharm.*, Vol. CLXXIII. (1874), p. 227.

$$NaH_2PO_4 + NaCl = Na_2HPO_4 + HCl.$$

As a fact, all four bodies will exist side by side during the reaction.

But not only is HCl formed by the interaction of dihydrogen sodium phosphate on chlorides, but likewise by the action of calcium chloride on hydrogen disodium phosphate, as shewn in the following equation:

$$3CaCl_2 + 2Na_2HPO_4 = Ca_3(PO_4)_2 + 4NaCl + 2HCl.$$

Maly thus believes that by the interaction of carbonic acid, disodic phosphate, monosodic phosphate, and sodic and lactic chlorides, hydrochloric acid is set free in the blood. But this acid possesses a higher diffusive power than any other acid, and we have only to surmise that the glands of the stomach are diffusion apparatuses of remarkable power in order to account for the separation of hydrochloric acid by them.

Objections to Maly's theory.

It is impossible not to appreciate the great value of the facts upon which Maly rests his theory, and not to acknowledge that he has thrown great light upon the part which diffusion may play in separating, from the alkaline blood, acids or acid salts which are present in it, although their reaction may be concealed by that of such salts as alkaline sodium phosphate. If, however, the reaction which results in the production of hydrochloric acid is one which goes on throughout the whole mass of the blood, and its separation occurs through a mere process of diffusion, we cannot but ask ourselves how it is that the acid only diffuses into the gastric juice, and not into the secretions of other glands which, as apparatuses of diffusion, offer obviously as favourable conditions as the stomach.

Why for instance does hydrochloric acid not pass into the urine? and unquestionably no trace whatever of any free mineral acid occurs in that secretion. Maly would meet the difficulty by attributing particular powers to the different glands as dialyzing apparatuses, but then we lose all the value of the physical explanations which appeared at first to be so likely to explain the phenomena.

Against the views of Maly there appear to be two capital objections. The first has reference to the chemical process which he supposes to occur in the blood, and to result in the liberation of free hydrochloric acid, and the second to the view that the glands of the stomach are from one point of view but apparatuses of diffusion which allow the hydrochloric acid of the blood to escape from it into the gastric juice.

Firstly—We can readily admit that there may coexist in the blood, acid and alkaline sodium phosphates and common salt, and that by the reaction of the latter on the former traces of hydrochloric acid may be formed, but it is impossible to admit that the latter acid would continue to exist side by side of sodium bicarbonate, with regard to the existence of which in the blood there cannot be a doubt.

Secondly—If the stomach acted as an apparatus of diffusion, separating, for instance, hydrochloric acid already formed in the blood, we should expect its secretion to be like that of the kidney (which is certainly the organ in which physical processes have most uncontrolled sway), a constant one, and to be influenced in a special manner by the condition of the blood and the general condition of the vascular system. The stomach, however, is a gland in which secretion only occurs as a result of the application of peculiar stimuli whose action unquestionably leads to the most remarkable changes in the secreting cells of the organ.

For these reasons we are forced to modify Maly's hypothesis so as to make it reconcileable with known facts.

The Author's modification of Maly's hypothesis.

We may conceive the acid-forming cells (*border-cells*) of the stomach to have, as cells of other secreting glands, peculiar selective powers in reference to certain saline constituents of the blood; we may conceive, for instance, of their possessing a peculiar selective affinity for the phosphates of sodium, both alkaline and acid, and for chlorides. This being granted, we have also to surmise that within the cell there occur the reactions which certainly do occur *in vitro* when the above salts coexist in solution; one of the products of the reaction will then be hydrochloric acid, which, in virtue of its high power of diffusion, will pass, as soon as formed, into the secretion of the gland.

In this hypothesis we remove the seat of the formation of hydrochloric acid from the blood generally to the gastric glands, and, whilst we adopt Maly's conception as to how physical and chemical processes may lead to the formation of the acid of the gastric juice, we subordinate them to the activity of the glandular epithelium, which must first bring together the bodies which have to react one upon the other.

Apart from the probability which attaches to the above hypothesis on theoretical grounds, it is to be noted that the only reliable analyses of the mineral constituents of the gastric juice, viz. those of Carl Schmidt, have proved that the gastric juice contains, in the main, in addition to its organic matters and its hydrochloric acid no inconsiderable quantity of mineral salts, which consist firstly of chlorides of sodium, potassium and calcium, and secondly of calcium and magnesium phosphate. These are salts which we should expect to be present if the reaction whereby hydrochloric acid is generated were such as the following:—

$$2Na_2HPO_4 + 3CaCl_2 = Ca_3(PO_4)_2 + 4NaCl + 2HCl.$$

The following are the results of Carl Schmidt's analyses of gastric juice, which are quoted in this place because of the bearing of the results of the analyses of the salts found, upon the views of the mode of production of the acid of gastric juice:

	Human.	Dog.		Sheep.
		1	2	
Water	994·40	973·06	971·17	986·14
Organic Matters, specially Pepsin, &c.	3·19	17·13	17·34	4·05
HCl	0·20 (?)	3·34	2·34	1·23
$CaCl_2$	0·06	0·26	1·66	0·11
$NaCl$	1·46	2·50	3·15	4·37
KCl	0·55	1·12	1·07	1·52
NH_4Cl	—	0·47	0·54	0·47
Precipitate by NH_3 $Ca_3(PO_4)_2$	0·125	1·73	2·29	1·18
$Mg_3(PO_4)_2$		0·23	0·32	0·57
$FePO_4$		0·08	0·12	0·33

Variations in the Proportion of Pepsin and Acid in the Gastric Juice.

Pepsin. The proportion of pepsin in the gastric juice undergoes considerable variation during the progress of digestion, undergoing first of all a diminution and then an increase. This variation is observed also in the case of the secretion of the isolated fundus, as has been shewn by Heidenhain, see Fig. 8, p. 105.

Acid. At the commencement of digestion the acidity of the gastric juice is less than subsequently, but this is not the case when the secretion of the isolated fundus is examined. The comparatively low acidity in the early periods is probably due to the acid reaction being neutralised by the alkaline saliva and by the alkaline secretion of the pyloric glands (Heidenhain). We shall return to this subject again.

SECT. 10. THE ACTION OF THE GASTRIC JUICE, AND ITS CONSTITUENTS, ON THE PROTEIDS.

The Researches and Views of Meissner.

The first of the systematic investigations on the products resulting from the digestion of proteids by pepsin and hydrochloric acid, was carried out by Meissner and his pupils between the years 1859 and 1862, and as subsequent writers have all more or less referred to, or employed certain of, the terms which he applied to the products which he obtained, a brief *résumé* of his chief conclusions appears desirable[1].

Parapeptone. This term was applied by Meissner to the neutralisation precipitate obtained when the product of digestion of a proteid by natural or artificial gastric juice is so nearly neutralised that only a faint acid reaction persists. Under these circumstances there falls a white flocculent precipitate, composed of the body to which Meissner ascribed the name of parapeptone. He described it as a body insoluble in pure water, but easily soluble in the weakest solutions of acids and alkalies, and precipitated from its solutions by the addition of sodium or potassium chloride. Parapeptone has been usually but incorrectly spoken of as identical with acid albumin or syntonin. It is identical with the body to which Kühne has assigned the name of antialbumat (see p. 121). In what respect, it will be asked, does parapeptone or antialbumat differ from syntonin or acid albumin? Apparently in the fact that whilst the latter body can readily be digested by pepsin and an acid, parapeptone is unacted upon by them. This remarkable property of undigestibility was pointed out by Meissner himself as distinguishing parapeptone from acid albumin. A further distinguishing property was pointed out by Meissner, to wit,—that when a solution of syntonin is nearly neutralised and pure alcohol is added, this throws down a precipitate. Under exactly similar circumstances a solution containing parapeptone is not precipitated. If the alcohol, however, contains ether, parapeptone is thrown down.

Metapeptone. On adding a little more acid to the liquid from which 'parapeptone' has been precipitated, Meissner obtained occasionally a further, though scantier, precipitate, separable by filtration, and insoluble in very dilute acids (0·1 per cent.) though soluble in stronger acids. This body Meissner termed 'metapeptone,' and looked upon also as an end-product.

Peptones α, β and γ. In the filtrate from which parapeptone and metapeptone had been separated, Meissner distinguished three separate soluble bodies, which he classed together in the group of peptones, but which he found to differ somewhat in their reactions.

Peptone α, precipitable by concentrated nitric acid as well as by potassium ferrocyanide and dilute acetic acid.

Peptone β, not precipitated by nitric acid, but by potassium ferrocyanide and strong acetic acid.

Peptone γ, not precipitated by nitric acid, nor by acetic acid and potassium ferrocyanide.

We now know that Meissner's peptones α and β were bodies which we term albumoses, whilst peptone γ corresponds to peptone properly so-called.

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[1] Meissner, *Zeitschrift für rat. Med.* Bd. VII. VIII. X. and XIV.

Dyspeptone.

On the prolonged digestion of casein or of fibrin, Meissner obtained a certain flocculent insoluble residue, to which he gave the name of *dyspeptone*. This body was insoluble in 0·2 per cent. HCl., but soluble in stronger acids. It consisted of a mixture of *antialbumid* (see p. 121) with nucleins.

Brücke's views on the Digestion of Proteids.

According to Brücke, when a typical proteid—unboiled fibrin—is subjected to peptic digestion, the following steps in the process occur. The fibrin is dissolved and, in great part, converted into acid-albumin or parapeptone, though even from the first some peptone is produced. At this stage a large precipitate is obtained on neutralising the liquid (Meissner's parapeptone). If digestion be very long continued, however (sometimes days are needed), the parapeptone disappears, so that no precipitate is obtained on neutralising[1], and the solution merely contains peptones.

The bodies taken for parapeptones by Brücke were, doubtless, in great part albumoses.

The Researches of Schützenberger[2].

Hemiprotein and Hemialbumin.

In the year 1885 Schützenberger published the results of a series of important researches on the products of decomposition obtained when albuminous substances are (*a*) subjected to long-continued boiling with dilute sulphuric acid, (*b*) digested in closed vessels at temperatures varying between 100° C. and 150° C. with solution of barium hydrate.

Under the influence of boiling dilute acid the proteid molecule is split up into two moieties, Hemiprotein and Hemialbumin.

Schützenberger placed a kilogramme of moist coagulated albumin, in 6-8 litres of water, to which 200 grams of H_2SO_4 has been added, and boiled this mixture between one-and-a-half and two hours. On allowing the mixture to cool, there separated a flocculent, homogeneous precipitate, resembling silica, or freshly precipitated magnesia. The latter, collected on a filter, washed, and dried, presented the appearance of yellowish transparent masses, which yielded a nearly white, not hygroscopic powder, insoluble in water, alcohol and ether. This substance was found by Schützenberger to amount approximately to one-half the weight of the albumin operated upon. To it he gave the name Hemiprotein. He found it to be amorphous, soluble in alkalies, from its solutions in which it could be precipitated on neutralising with acids, the precipitate being dissolved by an excess of acid.

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[1] In the filtrate from the precipitate there is, besides peptone, a soluble proteid which is coagulable on boiling.

[2] M. P. Schützenberger, 'Recherches sur l'albumine et les matières albuminoïdes.' *Bulletin de la Société Chimique de Paris.* Tomes (23) pp. 161, 193, 216, 242, 385, 433, (24) pp. 2 et 145.

These papers are abstracted at great length and with much ability in Maly's *Jahresbericht*, Bd. v. (1875), pp. 299—313.

The following are the results of elementary analyses of Hemiprotein, dried at 110° C.

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
C.	52·66	54·83	53·33	...	...	...	...	...
H.	7·01	7·25	7·31	...	...	...	...	...
N.	...	...	...	14·27	14·46	15·08	14·26	14·22

Hemiprotein contains sulphur, the amount of which has not been determined.

Hemiproteidin.

Schützenberger found that when hemiprotein was further subjected to a process of long boiling with dilute sulphuric acid, it furnished leucin and tyrosin, and as a chief product, an amorphous substance of a feebly sweet taste, soluble in water and alcohol, to which he gave the name of *Hemiproteidin*, to which as a result of his analyses, he assigned the empirical formula

$$C_{24}H_{42}N_6O_{12} + H_2O.$$

Hemialbumin.

Returning now to the original operation which yielded the insoluble hemiprotein, Schützenberger found that the acid filtrate from the latter contained, as its principal constituent, an amorphous substance, of feebly acid reaction, and containing approximately C 50%, H 7%, N 15·4%, to which he gave the name Hemialbumin, and the formula $C_{24}H_{40}N_6O_{10}$.

In addition to the two principal products of the decomposition of the proteid molecule by boiling dilute sulphuric acid, Schützenberger obtained evidence of the presence of many other interesting by-products, as for instance, a substance similar to, if not identical with, sarcine, a non-nitrogenous, strongly reducing, body apparently resembling glucose, and a dibasic acid, represented by the empirical formula $C_{24}H_{40}N_6O_{15}$.

Kühne's Observations and Theoretical Views.

Whilst Schützenberger was devoting his attention to the investigations some of the results of which have been referred to, Kühne had been studying the profound decomposition which the proteid molecule undergoes under the influence of *trypsin*, as he termed the powerful proteolytic enzyme of the pancreas and its secretion.

Whilst pepsin in acid solution and under suitable conditions of temperature, is able to convert a proteid into peptones, no further decomposition of these peptones occurs however prolonged the action of the artificial gastric juice, or however abundant the pepsin at work. Trypsin in alkaline solutions, on the other hand, was found by Kühne to decompose proteids in such a manner that there resulted, as final products of digestion,—a quantity of peptone which roughly amounted to half the weight of the proteid acted upon, together with a mixture of much less complex bodies, of which the best characterised were certain amido-acids, and particularly leucin (amido-caproic acid) and tyrosin (oxy-phenyl-amido-propionic acid).

Further, when the peptone resulting from the prolonged action of an artificial gastric juice was subjected to the action of trypsin, this enzyme was found capable of effecting the decomposition of a moiety, but a moiety only, of the peptone, and this yielded the same products as would have resulted from the direct action of trypsin on the original proteid.

These interesting results led Kühne to a surmise similar to that to which Schützenberger had arrived, and served as starting points of several investigations which resulted in the conception, which we owe to Kühne, of the general lines upon which the decomposition of a proteid proceeds under the influence of hydrolytic agents, however diverse.

To the peptone which resisted the action of trypsin, and which, owing to this characteristic, he had no difficulty in separating from the other products, Kühne immediately assigned the name of *Antipeptone*, whilst he assigned the name of *Hemipeptone* to a hypothetical peptone which he believed to be associated with antipeptone in the mixed products of the action of pepsin and gastric juice, and which yielded leucin and tyrosin when digested with trypsin although he had not, as yet, been able to separate it in a state of even approximate purity. To the mixed peptones resulting from the decomposition of proteids by pepsin and acid, Kühne has, since then, assigned the name *amphopeptones*.

The correctness of the surmise was, however, soon proved, and Kühne was able to describe the methods of preparing and separating, in approximate purity, the hemipeptone of which he had predicted the existence; further by comparing the decomposition of proteids under varied hydrolytic conditions, alone and in association with Chittenden, he was able to discover several interesting products, occupying an intermediate position between the native proteids and the peptones. These researches are so full of interest, and a knowledge of them is so essential to the investigator in all departments of Physiological Chemistry, that a somewhat detailed exposition of them is necessary[1].

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[1] The whole account which follows concerning albumoses and peptones is condensed from the various elaborate memoirs published on the subject by Kühne in association

Before examining the particular results to which Kühne arrived we may briefly express their general tendency as follows: every proteid when subjected to hydrolytic decomposition, or to conditions which presumedly bring about decomposition by causing the body in question to combine with the elements of water (such as boiling with dilute sulphuric acid: prolonged heating of dilute hydrochloric acid (0·25 per cent.) at 40° C.: digestion with pepsin and hydrochloric acid: digestion with trypsin in solution of sodium hydrate or sodium bicarbonate: putrefaction), splits up into bodies which belong to two distinct groups, a *hemi*-group, and an *anti*-group. We may conceive of the complex proteid molecule combining with the elements of water and breaking up into two (or more than two) molecules, of which one belongs to the *hemi*- and the other to the *anti*-product of decomposition. By the continued action of the hydrolytic agent, the primary cleavage products of each category are further decomposed, until, under favourable conditions, there result x molecules of antipeptone and of hemipeptone respectively. The bodies of the latter group are distinguished by their greater instability from those of the former group, and, particularly, by the fact that under the influence of trypsin they are capable of being decomposed into substances of comparatively simple constitution and of which the best characterised are leucin, tyrosin, and glutamic acid, whilst the bodies of the anti-group cannot, under the influence of trypsin and an alkali, be split up into bodies less complex than antipeptone. In the first paper[1] in which Kühne announced the views under discussion he exhibits the relation of certain of the bodies resulting from the decomposition of a proteid, by the aid of the subjoined schema, which shews the principal products of the action of hydrolytic agents on proteids. It will be observed that whilst the ultimate product of the splitting up are peptones, termed respectively *anti*-peptone and *hemi*-peptone, each of these is related to and derived from a so-called *albumose*. As will be shewn in the sequel, the best characterised of the intermediate products occupying a position between the native proteids and the peptones are the *albumoses*, *anti*- and *hemi*-albumose respectively—bodies which, whilst possessing some of the reactions of the true peptones, and particularly exhibiting the so-

Albumin

Anti-group	Hemi-group
Antialbumid (Hemi-protein)	
Antialbumat (Parapeptone)	
Antialbumose	Hemialbumose
Antipeptone	**Hemipeptone**

---

with his pupil Chittenden. The author has, in many places, quoted descriptions of methods and properties of substances almost verbatim.

[1] Kühne, 'Weitere Mittheilungen über Verdauungsenzyme und die Verdauung der Albumine.' *Verhandlungen des naturhist. med. Vereins zu Heidelberg.* Bd. I. Heft 4, S. 236.

called biuret-reaction (which had been supposed to distinguish the peptones from all other proteids), differ, it has been stated, from the peptones in possessing a lower power of diffusing through animal membranes, a distinction which, if it really existed, would establish the fact that they possess a larger molecular weight than the peptones. The statements which Funke originally made[1] concerning the high diffusibility of the peptones have, however, in no respect been confirmed by the investigations undertaken by von Wittich[2] in order to test them, nor by those of so competent and trustworthy an observer as the late Richard Maly[3].

Antialbumat and Antialbumid.

Products resulting from the decomposition of proteids by dilute acids. Antialbumat.

When proteid substances are digested at a temperature of 40° C. with hydrochloric acid containing 0·25 per cent. or boiled with dilute sulphuric acid (3—5 per cent.), or digested with an acid solution containing but a small quantity of pepsin, the decomposition is effected with the formation of considerable quantities of the body which Meissner termed parapeptone, but to which Kühne has assigned the new name *antialbumat*[4]. This body is precipitated from the solution by neutralising it. It is found, as Meissner had stated, to be soluble in dilute acids, as well as in a mixture of pepsin and acid; *when digested with the latter it is, however, unacted upon.*

Antialbumat is dissolved by an alkaline solution of trypsin, which at 40° converts it into antipeptone.

When treated for a long time with acids, antialbumat is converted into a much less soluble body, to which Kühne assigns the name *Antialbumid.* This formed part of the *dyspeptone* of Meissner, which contained, in addition, so-called *nucleins.* The substance to which Schützenberger assigned the name Hemi-protein is antialbumid.

Antialbumid.

As was said when reference was made to the body discovered by the French chemist, antialbumid is precipitable from acid solutions when these are neutralised; the precipitated body is wholly undigested by a mixture of pepsin and dilute acid; it is soluble in a weak alkaline solution, *e.g.*, in a weak solution of sodium hydrate.

The characteristic reaction which distinguishes antialbumid, from other bodies, is the jelly-like coagulation which its solution in sodium hydrate (containing 5°/₀) undergoes, when digested with trypsin; this coagulation resembles that produced by rennet in fluids containing casein. When the jelly is subjected to long-continued digestion with trypsin, it is in part gradually dissolved, and the solution is found to contain antipeptone, as well as some unchanged antialbumid, but no trace of leucin or tyrosin.

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[1] Funke, *Lehrbuch der Physiologie.* 5th ed. Vol. I. p. 208. Virchow's *Archiv*, Vol. XIII. (1858), p. 449.

[2] v. Wittich, 'Ueber die Diffusibilität der Peptone.' *Berl. klinisch. Wochenschrift*, 1872, no. 37. Abstract published in Maly's *Jahresbericht*, Vol. II. (for 1872), p. 19.

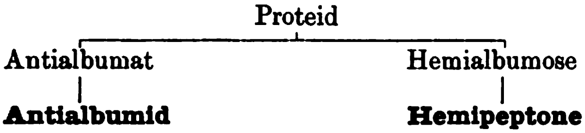
[3] Maly, 'Ueber die chemische Zusammensetzung und physiologische Bedeutung der Peptone.' Pflüger's *Archiv*, Vol. IX. (1874), pp. 565—619.

[4] Kühne, *Weitere Mittheilung über Verdauungsenzyme*, p. 5.

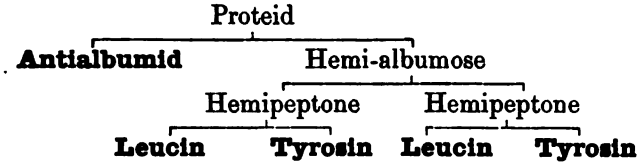
Whilst the action of acids gives rise, in the way described, to those special products of decomposition belonging to the anti-group, which are denominated antialbumat or antialbumid, there are also, necessarily, separated at the same time bodies of the hemi-group, and ultimately these are resolved, according to circumstances (nature and strength of acid, temperature and duration of process of heating) into hemipeptones or into the amido-acids which result from decomposition of the latter. The production, as well as the facts connected with the formation, of the bodies of the hemi-group, as well as the formation and properties of antipeptone are most suitably discussed in connection with the action of the enzymes on proteids. Before treating this branch of the subject, the schemata which Kühne has furnished exhibiting the decomposition of proteids under the influence of acids are placed before the reader.

Schema of Proteid Decomposition by Acids.

a. Action of dilute HCl (0·25 per cent.) at 40° C.



b. Action of dilute H_2SO_4 (3—5 per cent.) at 100° C.



The Albumoses.

Albumoses and peptones resulting from the action of pepsin and acids on proteids.

We have seen that under the influence of heat, dilute solutions of the mineral acids are able to effect the decomposition of the proteid molecule, giving rise after long-continued action to certain quantities of bodies of the *anti*-group which resist the further decomposing action of the acids, as well as to products derived from the *hemi*-moiety of the proteid molecule.

The decomposition, which acids effect with comparative slowness, proceeds with infinitely greater rapidity in the presence of pepsin and dilute acid at 40°C. If the quantity of pepsin be sufficient to confer intense digestive properties on the solution, the decomposition proceeds in such a manner that there are not produced from the anti-moiety of the molecule any antialbumat or antialbumid; were the latter body produced, inasmuch as it is incapable of further decomposition by pepsin and acid, it would persist to the very end of the digestive process, whereas it is found that under favourable circumstances the whole of the proteid acted upon may be converted into peptones.

By arresting the process of peptic digestion in its earlier stages, Kühne, as was previously stated, has separated from digestive mixtures bodies which are included under the generic name of albumoses, which are intermediate between proteids on the one hand and peptones on the other, there being certain of them which being convertible by a continued action of enzyme into antipeptone are termed antialbumoses, and others convertible in a similar manner into hemipeptones and termed hemi-albumoses.

In the first instance, Kühne discovered and described under the term of hemi-albumose the first product of hydrolytic decomposition, derived from the hemi-moiety of the proteid molecule. He identified it with a body discovered and described long ago by Bence Jones[1] as a constituent of the urine in a case of osteo-malacia, and which Kühne had himself also been able to separate from the urine in a similar case[2]. Subsequent researches, however, have led Kühne to discover that hemi-albumose is a mixed product, consisting of several proteid bodies, which have essentially the same percentage composition, and which have several important characters in common: all of which probably owe their origin to the combination of the elements of water with the proteid, and occupy positions intermediate between proteids and peptones.

Hemi-albumose, thus, is a mixture of the albumoses, belonging to the hemi-moiety of the proteid molecule, which are all convertible directly by pepsin and acid into hemipeptone, whilst ultimately furnishing, when digested with trypsin and alkali, or when boiled with sulphuric acid, leucin, tyrosin, glutamic acid, &c. According to certain subordinate characters, such as solubility in water and solutions of sodium chloride, Kühne and Chittenden have described proto-albumoses, hetero-albumoses, deutero-albumoses, and dys-albumoses[3].

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[1] Bence Jones, *Philosophical Transactions*, 1848. Part I. p. 55.

[2] Kühne, 'Ueber Hemialbumose im Harn.' *Zeitschrift für Biologie*. Vol. xix. p. 209.

Kühne and Chittenden, under heading 'Albumosen im Harn bei Osteomalacia' in Memoir 'Ueber Albumosen.' *Zeitschrift für Biologie*. Vol. xx. p. 40.

[3] Kühne und Chittenden, 'Ueber Albumosen.' *Zeitschrift für Biologie*. Vol. xx. (1884), pp. 11—51.

Kühne und Chittenden, 'Myosin und Myosinosen.' *Zeitschrift für Biologie*. Vol. xxv. (1889), pp. 358—368.

Chittenden und Hart, 'Elastin und Elastinosen.' *Zeitschrift für Biologie*. Vol. xxv. (1889) pp. 368—390.

Antialbumose.

It has been stated that when proteids are digested with dilute mineral acids, antialbumat and antialbumid are the only representatives of the anti-moiety of the proteid molecule. When Kühne had discovered in hemi-albumose an intermediate product between proteid and hemipeptone, he was naturally led to surmise that there probably existed an analogous albumose corresponding to antipeptone. Direct experiment furnished evidence which leaves no doubt, indeed, of the existence of such a body, though it has not been obtained in a condition of purity nor in large quantity. The substance is found in the precipitate obtained by neutralising digestive mixtures of acid pepsin and proteid, but, as follows from what has been already stated, in the neutralisation precipitate, any antialbumose which may be present is mixed with antialbumat.

The following account of one of Kühne and Chittenden's experiments, having for its object the separation of antialbumose, will convey an idea of the methods to be employed[1].

The albumin of 50 eggs was treated with water and acetic acid and coagulated by heat. The coagulum having been washed and freed from water, by pressure, was digested in two litres of dilute HCl (0·4 per cent.) at 30° C., mixed with one litre of artificial gastric juice at the same temperature.

The artificial gastric juice had been prepared by digesting for 48 hours at 40° C. the whole of the mucous membrane of a pig's stomach, with the exception of that from the pyloric region, in 2 litres of HCl containing 0·4 per cent. After this digestion the liquid had been dialysed for 24 hours in a Kühne's tube dialyser (see Fig. 7, p. 89), in a stream of running water: salicylic acid in the proportion of one part in 1000 having been added before dialysis, and hydrochloric acid being added at its conclusion so as to again bring the amount of acid to the original amount, viz. 0·4 per cent.

The digestion having proceeded for an hour and a half, the mixture was filtered, so as to separate the considerable quantity of yet undissolved albumin. This was treated with a fresh quantity of the gastric juice; in 15 hours at 40° C., solution was effected, the quantity of liquid amounting to 600 c.c. The solution thus obtained, after filtration, was neutralised, when a precipitate fell, considerable in amount, yet small in comparison to the amount of albumin operated upon. The precipitate was washed, dissolved in 150 c.c. of gastric juice, digested for 48 hours, and, on neutralising, again precipitated, not appreciably diminished in amount. In this way was obtained a body having the same characters as Meissner's parapeptone.

This body consisting of a mixture of antialbumose and antialbumid, was dissolved in solution of sodium hydrate (0·75 per cent.) and digested for about 48 hours at 40° C. with a dialysed pancreatic extract. No clotting occurred.

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[1] Kühne und Chittenden, 'Ueber Albumosen.' *Zeitschrift für Biologie.* Vol. xx. (1884).

From the solution after digestion, acetic acid added to neutralisation, threw down a substance having the characters of antialbumid, and particularly exhibiting the characteristic property of yielding when digested with soda and trypsin a thick jelly. The filtrate from the acetic acid precipitate of antialbumid contained antipeptone, which when digested during eight days at 40° C. with soda and trypsin gave no trace of leucin or tyrosin.

From the solution, antipeptone could be precipitated by concentration and the addition of alcohol.

Hemi-albumose and the Albumoses.

As the reader has gathered from much which has preceded, hemi-albumose is the name which was applied by Kühne to the first product of the action of hydrolytic agents and especially of the proteolytic enzymes, upon the hemi-moiety of the proteid molecule. To the same product the term *a peptone* had been assigned by Meissner and *propeptone* by Schmidt-Mülheim[1].

As a result of his earlier researches, Kühne announced hemi-albumose to be a body sparingly soluble in cold water, more soluble in hot water; precipitable by nitric acid, the precipitate being dissolved on adding an excess of acid, or on heating; the precipitate, in the latter case, returning when the liquid is allowed to cool. Hemi-albumose was found, further, to be soluble in weak solutions of NaCl, from which it is deposited if the salt be added to saturation. Acetic acid and ferrocyanide of potassium precipitated solutions of hemi-albumose. With copper sulphate and sodium hydrate, hemi-albumose was found to give in an intense degree the 'biuret' reaction, which had been supposed to be characteristic of the true peptones.

A further, most useful, character of hemi-albumose, a knowledge of which we owe to Wenz[2], is that of being completely precipitated when its solutions are saturated with ammonium sulphate. This salt affords indeed an easy and certain method of separating peptones from all other proteid substances in a digestive mixture, these being thrown down, whilst the peptones are left in the solution, which then may be freed from the ammoniacal salt by dialysis, or by boiling with barium carbonate or hydrate.

Even in one of his earlier papers, on the immediate products of decomposition of the proteids, Kühne had expressed a doubt as to the product which he had termed hemi-albumose being a definite chemical individual. The researches, which he in conjunction with Chittenden afterwards carried out, proved conclusively that the hemi-albumose which he had first obtained, is a mixture of several bodies, to which they have assigned the names 'proto-albumose,' 'deutero-albumose,' 'hetero-albumose' and 'dys-albumose.' Still it appears to the Author that we may with advantage retain the term *hemi-albumose* for the mixed product containing all the albumoses.

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[1] Schmidt-Mülheim, 'Untersuchungen über d. Verdauung der Eiweiss-Körper.' Du Bois Reymond, *Archiv f. Anat. u. Phys.*, Phys. Abth. 1879, p. 1.

[2] Wenz, 'Ueber das Verhalten der Eiweissstoffe bei der Darm-Verdauung.' *Zeitschrift f. Biologie.* Bd. 22 (1886), S. 1.

Method of obtaining 'hemi-albumose' (i.e. the mixed albumoses) from fibrin.

Blood fibrin affords a material from which the mixed albumoses can be obtained with the greatest ease.

Fibrin obtained by stirring or whipping blood is thoroughly washed with water and it is then placed in 0·2 per cent. HCl until it has swollen up into a jelly; the acid and fibrin are heated to 37° and an active solution of pepsin is then added, which causes solution of the fibrin to occur in the course of very few minutes. Digestion may be allowed to go on for about an hour and may then be stopped either by cooling the liquid or, better still, by neutralising it with sodium hydrate.

Kühne and Chittenden in one of their experiments placed 1500 grammes of raw fibrin in 5 litres of HCl (0·4 per cent.) for 24 hours, then heated it to 45° C. and mixed with it 400 c.c. of normal artificial gastric juice and digested the mixture for 1 hour[1].

An abundant precipitate falls which is separated by filtration, and to the filtrate powdered sodium chloride is added in such quantities that some remains undissolved and mixed with the abundant white precipitate, which is collected in a filter and washed with a little saturated NaCl solution. The product is hemi-albumose, i.e. a mixture of the albumoses derived from the hemi-moiety of the proteid molecule.

Method of separating the various 'albumoses' from the mixed 'hemi-albumose.'

The product described in the preceding paragraph is separated from the filter and is pounded in a mortar together with a saturated solution of NaCl, which dissolves and holds in solution the so-called deutero-albumose, whilst proto-albumose, dys-albumose and hetero-albumose remain undissolved.

From the solution in NaCl above referred to deutero-albumose is precipitated by means of acetic acid in which sodium chloride has been dissolved.

The separation of the albumoses thrown down by NaCl is effected as follows. The mixed precipitate containing them is pressed so as to free it as thoroughly as possible from adhering salt solution, and is then treated with a considerable quantity of water, which dissolves it almost entirely. The insoluble matter is separated by filtration and ground in a mortar with 5 per cent. solution of NaCl, and then with water, and constitutes the so-called dys-albumose. The united solutions containing NaCl are dialysed, whereupon a precipitate occurs of so-called hetero-albumose, whilst proto-albumose is left in solution.

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[1] All the details, a knowledge of which is necessary for repeating Kühne's experiment, will be found in the paper by Chittenden and himself, 'Ueber Albumosen,' *Zeitschrift für Biologie*, Vol. xx. (1884), pp. 11—51: refer to p. 13. The reader may also refer to a previous paper by the same authors, 'Ueber die nächsten Spaltungsprodukte der Eiweiss-Körper' *Zeitschrift für Biologie*, Vol. xix. (1883), pp. 159—208: refer to pp. 184 and 185.

Proto-albumose. The aqueous solution which has been dialysed and thus separated from heteroalbumose, possesses, as do, according to Kühne, all solutions of albumoses, an alkaline reaction.

In order to obtain proto-albumose in a state of purity, the solution is saturated with NaCl, the precipitate dissolved in water, and the process of precipitation and solution repeated four times.

From the watery solution of this purified proto-albumose, the solid substance is obtained by precipitating directly with alcohol, or by first concentrating the solution in the water bath and then adding alcohol to the concentrated solution. The alcohol precipitate is washed successively with alcohol and ether, after treatment with which, proto-albumose is obtained in thick, snow-white masses. The substance is highly soluble, and although its solution is not effected immediately, the quantities dissolved are so great, that a solution of syrupy consistence can be obtained. The solutions are colourless, not perfectly, although almost perfectly, transparent, and have an alkaline reaction.

When nitric acid is added, drop by drop, to a pure solution of proto-albumose, a white precipitate falls which at first disappears when the fluid is mixed by shaking, a fresh precipitate occurring on addition of more acid. The precipitate which persists after adding a sufficient quantity of acid, disappears on heating, but is deposited again as the liquid cools. An excess of nitric acid, even in the cold, dissolves the precipitate at first formed, the solution assuming a yellow colour; sodium chloride added to it throws down a precipitate which is entirely dissolved by heat, and deposited again on cooling.

Solutions of proto-albumose are precipitated by acetic acid and potassium ferrocyanide. If the quantity of acetic acid be small the precipitate is undissolved by heat; if, on the other hand, strong acetic acid be added to the solution, a larger quantity of ferrocyanide is required to produce a precipitate, but this is dissolved on heating the liquid, which becomes turbid on cooling.

Solutions of proto-albumose if saturated with NaCl and filtered, become a little less transparent when boiled, and the turbidity continues when they are cooled. The addition of a trace of acetic acid to such solutions causes them to become very turbid.

Cupric sulphate, mercuric chloride, and basic lead acetate, cause heavy precipitates, which in the case of the first and third of these reagents are soluble in excess of the precipitant. These precipitates are only partially dissolved when the liquid in which they occur is boiled.

Treated with a trace of copper sulphate and a large quantity of solution of sodium hydrate, solutions of proto-albumose exhibit the beautiful red-violet so-called 'biuret' reaction. When boiled with lead acetate and alkali, solutions assume a dark colour due to the formation of PbS_2.

Proto-albumose is laevogyrous. Kühne and Chittenden determined the specific rotation of five different preparations of the substance and obtained the following results:

Preparation A $(\alpha)_D = -72^\circ{\cdot}64$
B $(\alpha)_D = -79^\circ{\cdot}05$
C $(\alpha)_D = -77^\circ{\cdot}90$
D $(\alpha)_D = -73^\circ{\cdot}18$
E $(\alpha)_D = -71^\circ{\cdot}40$.

The following are the means of several analyses of each of five different preparations of proto-albumose (Kühne and Chittenden).

Preparation	A (Mean)	B (Mean)	C (Mean)	D (Mean)	E (Mean)
Carbon	50·89	50·39	50·54	51·50	50·55
Hydrogen	6·83	6·74	6·69	6·80	6·85
Nitrogen	17·12	17·12	17·34	17·13	17·01
Sulphur	1·17	1·07	1·17	0·94	1·07
Oxygen	23·99	24·68	24·26	23·63	24·52.

Hetero-albumose. As has been already stated, this body separates from the salt solution of the mixed albumoses, during dialysis, and adheres as a gum-like mass to the walls of the tube dialyser in which the operation is carried on, so that in order to separate it the tubes must be cut open. Hetero-albumose cannot consequently be separated from the proto-albumose solution by filtration, but its collection is rendered more easy if the liquid be saturated with sodium chloride, after which it is separated by decantation. The crude hetero-albumose, thus obtained on drying, assumes the appearance of plates of gelatin. In order to purify the substance, two methods are available; the first of which consists in dissolving it in 5 or 10 per cent. solution of NaCl, and simply dialysing the solution; the second consists in precipitating the salt solution just referred to by means of rock salt, redissolving the precipitate, and then dialysing the solution.

Hetero-albumose is insoluble in pure water, which thereby causes fragments of the substance to soften and swell. Solutions of NaCl, so dilute as to contain less than 0·5 per cent. of the salt, or strong to complete saturation, dissolve hetero-albumose, though slowly, in considerable proportions, though the salt acts most efficiently as a solvent in proportions varying between 5 and 10 per cent. On completely saturating solutions of hetero-albumose with NaCl, the substance is abundantly precipitated, though precipitation is not complete, and on subsequent dialysis, a further precipitate is obtained. Strong solutions in 5 to 10 per cent. solutions of NaCl are rendered turbid by the addition of water.

In addition to neutral salts, dilute solutions of acids, alkalies, and alkaline carbonates, act as efficient solvents of hetero-albumose.

The solutions, thus obtained, are, in general, precipitated by neutralising them, though never completely so, as the substance is held in solution by the salts which are formed during the process. NaCl solutions of hetero-albumose always have a distinctly alkaline reaction.

The most characteristic property of hetero-albumose is the behaviour of its solutions when boiled, as well as the peculiar behaviour of the undissolved body when the fluid in which it is suspended is boiled.

When hetero-albumose is suspended in water and boiled, it becomes completely insoluble in solutions of NaCl of all strengths. During the process of heating the substance appears to melt, and adheres in patches to the glass; on cooling, it sets in masses, which are either leathery or harder.

Saturated solutions of hetero-albumose, containing ½—2—3—5 per cent. of NaCl, coagulate about equally, *e.g.* the turbidity produced is about the same in degree in the different cases. If, however, the solutions have been diluted with from three to five times their own volume of solution of pure NaCl, containing the same amount of the salt as they contain, no precipitate is obtained when the liquid is boiled or when it subsequently cools. The gradual addition of acetic acid to these clear solutions causes, however, a turbidity, which increases at first, but after a certain point disappears. Nitric acid added to the fluid will now cause a fresh turbidity, which disappears on adding a sufficient excess of acid. If to the cold solution, which is clear and remains clear when boiled, strong solutions of NaCl be added, a precipitate occurs, which disappears, or nearly so, on boiling, and reappears again on cooling.

When to a solution of hetero-albumose only so much acetic or nitric acid, respectively, is added, as will occasion a perceptible turbidity, this is observed to increase as the liquid is heated, to disappear when it is boiled, and to reappear in increased degree, when it is cooled.

Hetero-albumose, which has been suspended in water and rendered insoluble by boiling the water, is found to swell and dissolve completely in HCl containing between 0·1 and 0·2 per cent., but to be almost insoluble in solutions of sodium hydrate (containing from 0·25 to 3 per cent. of NaHO). By its solution in hydrochloric acid the coagulated substance appears to have been in part reconverted into normal hetero-albumose, identical with the original substance, but in part to have been changed into a substance identical with dys-albumose.

Acetic acid and potassium ferrocyanide render solutions of hetero-albumose strongly turbid, the turbidity disappearing on adding a sufficient quantity of the acid. When the reagents are added in certain favourable proportions, it may be noticed that the turbidity occasioned by acetic acid and potassium ferrocyanide disappears on boiling the liquid, but reappears when it becomes cold.

With copper sulphate and sodium hydrate, hetero-albumose gives the biuret reaction, but it is to be noted that a very faint excess of the copper salt suffices to conceal entirely the rose colour, which is then covered by the well-known violet colour, characteristic of the albuminous substances in solution.

When boiled with lead acetate and sodium hydrate, a brown precipitate occurs in solutions of hetero-albumose, but this is smaller in amount than would be expected from the amount of sulphur which the substance contains.

Copper sulphate, and neutral and basic lead acetates, precipitate solution of hetero-albumose in sodium chloride, and the precipitates are insoluble in excess of the precipitants.

Unlike proto- or deutero-albumose, hetero-albumose in solution is not precipitated by mercuric chloride, whether the reaction be alkaline, neutral, or faintly acid. On addition of acetic acid, however, a precipitate falls which requires a very large excess of acid to dissolve it.

Hetero-albumose is laevogyrous, its specific rotation having been found (the mean of three determinations) to be $(\alpha)_D = -68^{\circ}\cdot65$.

The following is the composition of hetero-albumose, according to the analyses of Kühne and Chittenden:

Carbon	50·74
Hydrogen	6·72
Nitrogen	17·14
Sulphur	1·16
Oxygen	24·24

From these numbers it would appear that there is no appreciable difference in ultimate composition between proto- and hetero-albumose.

Deutero-albumose. This body, although present in the mixed 'hemi-albumose' product of digestion with pepsin and acid, was obtained by Kühne most easily from 'Witte's Peptone,' which is an article of commerce, and like the majority of other commercial preparations sold under the name of Peptones, contains large quantities of the mixed albumoses in addition to hemi- and anti-peptones.

The mixed albumoses, or Witte's peptone, having been pounded in a mortar with saturated solution of NaCl, and the solution having been filtered, it is treated with acetic acid, which at once throws down an abundant precipitate of deutero-albumose, which is collected in a bag, washed with saturated solutions of NaCl, redissolved again in weak salt solution, and reprecipitated with acetic acid. The precipitate is again dissolved, the salt and acetic acid are separated by dialysis, the acid with considerable difficulty, and best after having been cautiously neutralised with solution of sodium hydrate. In the dialyser, the hetero-albumose is found in solution. The latter is concentrated by evaporation, and the hetero-albumose is precipitated by the addition of alcohol; the precipitate is washed with absolute alcohol and ether and obtained as a perfectly white, light powder, which but for containing a small quantity of calcium sulphate (0·68—1·77 per cent.) appears to be perfectly pure.

Deutero-albumose is not precipitated from its solutions by saturation with sodium chloride.

Even when its solutions are saturated with NaCl and boiled no precipitation occurs.

Solutions in pure water are not precipitated by boiling, even after being acidulated with acetic acid. Nitric acid produces neither turbidity nor precipitate; when added in slight excess the liquid becomes yellow, even in the cold. If, however, sodium chloride be added in sufficient quantity to this yellow liquid it becomes turbid, but when heated, long before it reaches the boiling-point, it becomes clear; on cooling, however, the solution becomes as opaque as before.

When solutions of deutero-albumose are rendered feebly acid by means of acetic acid, and then a few drops of a solution of NaCl added, they may remain perfectly clear; on then gently heating, a turbidity often becomes visible, to disappear when the temperature rises, and to reappear permanently on cooling, after which the liquid must be heated almost to boiling-point in order to clear it.

If the quantity of NaCl added be gradually increased, however, it will be found that on boiling the solution, the precipitate almost, but never quite, disappears on boiling. If the liquid be filtered, however, it will be found, that though it at first passes through the filter perfectly clear, it at once commences to precipitate, in consequence of the sudden, though slight, fall in temperature. If acetic acid be added, however, to a cold NaCl solution, in which a precipitate had been caused, in quantity sufficient to dissolve the precipitated deutero-albumose, no further precipitation will be caused, though the liquid be again boiled.

When heated with lead acetate and sodium hydrate, deutero-albumose darkens intensely. To acetic acid and potassium ferrocyanide, as well as to solutions of neutral and basic lead acetate, copper sulphate, and mercuric chloride, deutero-albumose behaves precisely as proto-albumose.

Like all the albumoses it is precipitated completely from its solutions when these are saturated with neutral ammonium sulphate.

Deutero-albumose gives with copper sulphate and sodium hydrate, the rose colour which constitutes the biuret reaction.

Deutero-albumose is laevogyrous. The specific rotation of the preparation was found by Kühne and Chittenden to be (1) $(\alpha)_D = -74^\circ{\cdot}41$, and (2) $(\alpha)_D = -79^\circ{\cdot}11$.

The following numbers exhibit the composition of deutero-albumose:

Carbon	50·84
Hydrogen	6·85
Nitrogen	17·14
Sulphur	1·07
Oxygen	24·10

Dys-albumose. That portion of the mixed albumoses which is undissolved by saturated solution of NaCl, as well as by solutions containing respectively 10 and 5 per cent. of salt, is treated with water, and the insoluble matter is treated with dilute HCl (0·2 per cent.), in which it is for the most part soluble. The solution is filtered and the filtrate neutralised. By this treatment a considerable portion of the substance has become soluble in NaCl, and has in reality been converted into hetero-albumose; that portion which has not been converted into this substance and which has been precipitated by neutralisation, is thoroughly washed with solution of NaCl and water, and then extracted with alcohol and ether.

When dys-albumose is dissolved in sodium hydrate containing 1 per cent., on neutralising, it is found to have been almost entirely converted into a substance soluble in NaCl, precipitable from its NaCl solution with all the characters of hetero-albumose.

Similarly, when pure hetero-albumose is precipitated from its solutions by an excess of NaCl, a portion of it always appears to be converted into a body identical with dys-albumose, and the same change occurs when hetero-albumose is long preserved under alcohol, or in a dry condition.

On ultimate organic analysis, Kühne and Chittenden found dys-albumose to have the following composition:

Carbon	50·88
Hydrogen	6·89
Nitrogen	17·08
Sulphur	1·23
Oxygen	23·92

From the facts observed by them relating to the conversion of hetero-albumose into dys-albumose and the converse, Kühne and Chittenden draw the conclusion that dys-albumose is but hetero-albumose which has been converted into an insoluble modification through the agency of neutral salts.

The annexed table exhibits the mean of all the results of Kühne and Chittenden's analyses of the albumoses, as well as of their determinations of the specific rotatory power of each.

Neumeister's views concerning the albumoses. According to Neumeister the soluble albumoses should be divided into (1) primary albumoses which include proto-albumose and hetero-albumose and (2) secondary albumoses, viz., so-called hemi-deutero-albumose and ampho-deutero-albumose; the primary, representing a first stage of hydration, and the secondary a subsequent hydration.

Secondary albumoses are, according to Neumeister, distinguished from the primary by the following reactions:—(1) they are not precipitated by nitric acid from solutions which are free from saline

TABLE EXHIBITING RESULTS OF THE ANALYSES OF THE ALBUMOSES (KÜHNE AND CHITTENDEN).

	Proto-albumose.			Proto-albumose.		Deutero-albumose.		Hetero-albumose.	Dys-albumose.
				Precipitated by NaCl.	Precipitated by Acid.				
Designation.	A	B	C	D	E	F	G	H	J
Carbon Hydrogen Oxygen Nitrogen Sulphur	50·89 6·83 17·12 1·17 23·99	50·39 6·74 17·12 1·07 24·68	50·54 6·69 17·34 1·17 24·26	51·50 6·80 17·13 0·94 23·63	50·55 6·85 17·01 1·07 24·52	50·47 6·81 17·20 0·87 24·65	50·84 6·85 17·14 1·07 24·10	50·74 6·72 17·14 1·16 24·24	50·88 6·89 17·08 1·23 23·92
	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00	100·00
Ash per cent. $(\alpha)_D$	0·90 – 72°·64	0·22 – 79°·05	2·58 – 77°·90	1·60 – 73°·18	1·32 – 71°·40	1·77 – 74°·41	0·68 – 79°·11	0·90 – 68°·65	1·27

admixtures: (2) nor by a 2 per cent. solution of copper sulphate: (3) nor when their neutral solutions are saturated with sodium chloride. (4) Phospho-molybdic and phospho-tungstic acids precipitate the primary albumoses completely, but the secondary incompletely (?) According to Neumeister, hetero-albumose is related to *anti-* as well as to the *hemi-* moiety of the proteid molecule; by a subsequent hydration he supposes it to yield an ampho-deutero-albumose, i.e., a deutero-albumose in which both hemi- and anti-molecules are still present and which on final hydration will yield both *hemi-* and *anti*-peptone[1].

Classification of albumoses according to their origin.

The researches of Kühne and Chittenden, of Neumeister and others, have shewn that, corresponding to each of the principal proteids and also corresponding to several of the albuminoid substances, by the action of hydrolytic agents and especially of the digestive enzymes, bodies can be obtained which agree in their relations, and resemble for the most part very closely both in physical properties and chemical characters, the albumoses which have been described. Terms have been devised to designate these bodies, which serve to indicate their origin. Thus Kühne and Chittenden have described globuloses[2]; Chittenden and Painter caseoses[3]; Neumeister vitelloses[4]; Kühne and Chittenden myosinoses[5]; Chittenden and Hart elastoses[6]—these being the albumoses corresponding to globulin, casein, vitellin, myosin and elastin respectively. Similarly proto- and deutero-albumoses corresponding to the several bodies have received the names of proto-globulose, deutero-globulose, proto-myosinose, deutero-myosinose, &c.

It has been suggested, by Halliburton[7], that the albumoses, conjointly, should be designated *proteoses*. It appears to the author that no ground whatever exists for adding a fresh name to the cumbersome list of those already suggested, especially as the term proteose is non-suggestive of the origin of the bodies to which it is desired to apply it, and is certain to mislead: inasmuch as the Greek numeral adjectives having served as prefixes to distinguish the different albumoses, the suggested term proteoses is likely to be assumed, by many, to refer to the proto-albumoses, as distinguished from the deutero or hetero-albumoses, rather than as distinguishing the albumoses from the true peptones.

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[1] Neumeister, *Zeitschrift für Biologie*, Vol. xxvi. p. 57.

[2] Kühne and Chittenden. *Zeitschrift für Biologie*, Vol. xxii. p. 409.

[3] R. H. Chittenden and H. M. Painter, 'Casein and its primary cleavage products.' *Studies from the Laboratory of Physiological Chemistry of Yale College* for 1885—1886, p. 156.

[4] R. Neumeister, 'Ueber Vitellosen.' *Zeitschrift für Biologie*, Vol. xxiii. p. 2.

[5] Kühne and Chittenden, 'Myosin und Myosinosen.' *Zeitschrift für Biologie*, Vol. xxv. (1889) p. 358.

[6] R. H. Chittenden, u. A. J. Hart, 'Elastin und Elastosen.' *Zeitschrift für Biologie*, Vol. xxv. (1889), p. 368.

[7] Halliburton, 'A text book of Chemical Physiology and Pathology.' London, Longman and Co., 1891.

PEPTONES.

Whatever the intermediate products, the ultimate products which result from the action of gastric juice, natural or artificial, are bodies which, as stated previously, were first designated peptones by Lehmann. We have pointed out that Meissner distinguished several peptones, which differed in certain reactions one from another. Until lately, the characteristic reaction upon which general reliance was placed, in determining whether a body was a true peptone or not, was the so-called '*biuret*' reaction, i.e. the production of a rose-red colour with a minute quantity of solution of copper sulphate, followed by a very large excess of sodium or potassium hydrate.

The researches of Kühne have, however, revealed the fact that the biuret reaction is not distinctive of the true peptones, being shared by the albumoses, though the several bodies belonging to this class do not exhibit the reaction in an equally distinct manner, the slightest excess of copper leading in the case of hetero-albumose to the production of the violet colour which all the native albumins and their compounds exhibit, and which masks the rose colouration at first shewn. Other facts, observed chiefly by Kühne and Chittenden, have demonstrated that Meissner's α and β peptones consisted of albumoses.

This being the case, the question arises: how shall we distinguish the bodies to be termed true peptones? Although the distinction which, following the example of Kühne and other recent writers and investigators on this subject, we shall draw may be considered by some arbitrary, yet it appears convenient and correct.

It has been found[1] that ammonium sulphate added, to complete saturation, to solutions of albuminous substances which have been first of all neutralised and then rendered very faintly acid by a trace of acetic acid, precipitates all proteids including the several albumoses, with the exception merely of the bodies which we shall designate as peptones, which are left in solution.

Some[2] appear to think a distinction, based upon the action of a single reagent, artificial and unphilosophical, and, indeed, were this the case, it would be impossible to defend it. But as a matter of fact, the distinction does not rest upon the action of a single reagent.

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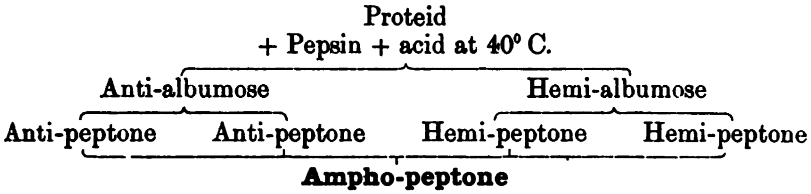
[1] J. Wenz, 'Ueber das Verhalten der Eiweissstoffe bei der Darmverdauung.' *Zeitschr. f. Biologie*, Vol. XXII. (1886), see p. 10. Heynsius had recommended ammonium sulphate as a general precipitant, not only for the native albumins and their derivatives, but for propeptone and peptone. It would appear from Wenz's observations, however, that Heynsius can only have experimented with albumoses and not with true peptones, as the latter were found not to be precipitated by the ammoniacal salts.

[2] Hammarsten, *Lehrbuch d. physiologisch. Chemie*.

It is found that the proteid matters which remain after precipitation with ammonium sulphate, and to which it is now proposed to restrict the term peptones, differ from all other bodies, even from the most closely related of the albumoses, by the most important property which they present, of being able to diffuse with much greater readiness through membranes, and especially through animal membranes. This property is obviously of the highest importance from a physiological stand-point, but it is no less so from a physical and chemical point of view, for it establishes, as we have already pointed out, the fact that the true peptones are bodies which differ from proteids in general, and from the albumoses in particular, in possessing a smaller molecular weight:—that they are certainly products of decomposition, and that whether it be true or false that the changes brought about by the digestive enzymes are of the nature of *hydrolytic* decompositions, they are certainly of the nature of decompositions, under the influence of which the giant molecules of the proteids are resolved into molecules so much smaller that they are able to find their way through the interstices of the tissues, and be absorbed. The above considerations warrant us, then, in establishing a distinction between peptones and those bodies which possess many points of resemblance to them.

It now remains for us to consider the special facts with which we are acquainted relating to the mixed peptones which result from the action of gastric juice, as well as to refer to the history, so far as it is known to us, of the individual peptones. To the mixed peptones, hemipeptone and antipeptone, Kühne and Chittenden[1] applied the convenient term *ampho-peptone*, which designates its dual character. Modifying slightly the original scheme suggested by Kühne[2] the production of ampho-peptone by the action of gastric juice upon proteids is shewn as follows:



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[1] Kühne, 'Weitere Mittheilungen,' &c., p. 9.

[2] "We have designated as ampho-peptone the end product of the digestion of albumin by pepsin and acid." 'Peptone,' by W. Kühne and R. H. Chittenden. *Studies in the Laboratory of Physical Chemistry of Yale University*, Vol. II. p. 15.

Methods of Preparation of Ampho-peptone.

It appears desirable to describe in the first instance the methods which have been employed in preparing the mixed peptones, by several investigators of repute, and then those modifications will be pointed out in them which the progress of research has suggested.

Henninger's method of preparing peptones.

Henninger[1] has made careful researches on the peptones obtained by the peptic digestion of fibrin, albumin and casein, employing the following method of preparation.

The proteid substance, whose peptone was required, was purified as completely as possible and was then digested with five times its weight of a solution containing 0·3 per cent. of sulphuric acid, together with pepsin, the temperature being maintained at about 44° C. After digestion had gone on for some time an additional quantity of dilute acid was added, and the digestion continued for three or four days, after which the sulphuric acid which the fluid contained was exactly precipitated with barium hydrate and the filtrate from barium sulphate was concentrated to a syrupy consistence on a warm bath.

The syrupy liquid was first of all treated with alcohol containing some water; this precipitated some peptone and a large proportion of the colouring matter present. The fluid was now poured, in a thin stream, into absolute alcohol, when the peptone was precipitated; it was redissolved in water, and again treated with alcohol containing water, and lastly with absolute alcohol, by which it was thrown down free from colour. The solid was then extracted with ether.

Herth's method.

The method employed by this observer, in preparing peptones of egg-albumin[2], appears to possess considerable merit.

The whites of 50—60 hard-boiled eggs were reduced to a fine state of division and then digested, during 24—30 hours, in a solution containing 1 per cent. of phosphoric acid; they were then separated from this solution and extracted with hot water, the object being to get rid as much as possible of earthy salts which had been rendered soluble by the digestion in acid. The egg-albumin was then placed in 4 litres of a solution containing 0·65 per cent. of H_3PO_4, and to this 40 c.c. of a clear dialysed solution of pepsin was added; the temperature was now raised to 40° C. In about five hours, the whole of the albumin had dissolved, though the process was continued for many hours more. The liquid was now heated on a sand-bath, and freshly precipitated, well-washed $PbCO_3$ was added, until the clear yellow liquid had a perfectly neutral reaction to litmus paper, and gave no longer the reactions of phosphoric acid, this having been removed as $Pb_3(PO_4)_2$. The solution contained a small quantity of lead, but so little that 100 c.c. of aqueous solution of sulphuretted hydrogen sufficed to precipitate it. After separating the lead in this way, the liquid was concentrated on the water-bath, precipitated with, and afterwards digested in, strong alcohol, again dissolved in water and re-precipitated with alcohol, the process being repeated three times in succession.

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[1] Henninger, 'De la nature et du rôle physiologique des peptones.' *Comptes Rendus* (1878), Vol. LXXXVI. p. 1413 and 1464.

[2] Herth, 'Ueber die chemische Natur des Peptons und seine Verhältniss zum Eiweiss (aus dem Lab. von Prof. Maly).' Hoppe-Seyler's *Zeitschrift f. phys. Chemie*, Vol. I. (1877), p. 277.

Kühne and Chittenden's method of preparing Ampho-peptone from Fibrin[1].

3800 grams of washed, but not boiled, fibrin were digested in two litres of a solution of hydrochloric acid containing 0·4 per cent. of HCl, with which had been mixed a purified pepsin obtained from 1220 grams of isolated mucous membrane of the fundus of the stomachs of two pigs. The mixture was allowed to remain at 37°—40° C. for a fortnight, so as to ensure as completely as possible the conversion of albumoses into peptones. At the end of that time filtered portions of the solution gave only slight precipitates on neutralisation, but a heavy precipitate was obtained with ammonium sulphate, with sodium chloride, with sodium chloride and acetic acid, and still further with sodium chloride and nitric acid and with metaphosphoric acid. Nevertheless the filtrate saturated with ammonium sulphate contained much peptone.

In order to separate and purify the peptone present in the filtrate the following processes were carried out:—the filtrate was neutralised with sodium hydrate, filtered through linen, with the special object of removing the impurities of the fibrin, the filtrate slightly acidified with acetic acid, concentrated to about 4 litres, precipitated with an excess of ammonium sulphate, filtered and pressed, the solution boiled with barium hydrate, and finally with barium carbonate and a large quantity of water, until ammonia could no longer be detected. The barium sulphate was then removed by filtration through linen bags which were repeatedly washed and pressed, the solution evaporated to about 4 litres, the barium peptone decomposed with a very slight excess of sulphuric acid, the new precipitate of barium sulphate filtered off, the solution concentrated to 2 litres, the pure acid neutralised with ammonia and, after cooling, 6 per cent. of English sulphuric acid, previously diluted, was added. The sulphuric acid-peptone solution was then precipitated with a large excess of phospho-tungstic acid, the precipitate washed first with 6 per cent. sulphuric acid and afterwards with a large quantity of water, after which the compound was decomposed by means of excess of barium hydrate, and the excess completely removed from the filtrate, by adding sulphuric acid. The peptone solution, thus obtained, had a distinctly acid reaction and, strange to say, contained hydrochloric acid, which was hardly to be expected after the very careful washing which the precipitate had received.

The solution was neutralised with ammonia, to render the acid harmless on concentration. Then by repeated precipitation, and boiling, with alcohol, some peptone was obtained, which was free from ammonium chloride.

The peptone, prepared in this way, was most difficult to obtain in a dry state, though it was ultimately converted into a fine exceedingly hygroscopic powder, by long heating *in vacuo*, at 105° C. In order to effect the drying, the alcohol was first displaced by boiling with water.

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[1] Kühne und Chittenden, 'Ueber die Peptone.' *Zeitschrift f. Biolog.* Vol. XXII. (1886), p. 425.

Distinguishing Characters of Peptones.

Physical character.

However different the proteids which are employed in their preparation, peptones all possess, essentially, the same physical and chemical properties.

When freshly precipitated and moist, pure peptones are white, resembling freshly precipitated casein; if the yet moist mass be heated to between 80° and 90° C., it has a tendency to melt into a fat-like mass which solidifies on cooling (Adamkiewicz and Hoffmann). Dried peptones appear as brittle, yellowish-white, solids, soluble in any proportion in cold or hot water, and from their great affinity for water, highly hygroscopic. They are insoluble in absolute alcohol, in ether, chloroform, and hydrocarbons: they dissolve in aqueous alcohol, according to the amount of water which it contains. The aqueous solutions of peptones have a neutral reaction, rotate the plane of polarization to the left, and possess a higher diffusive power than other soluble proteids; to this character, attention will again be directed.

Non-precipitation by heat and nitric acid.

When solutions of peptones are pure they are not precipitated when boiled, nor on the addition of any mineral acid. Adamkiewicz has asserted that this statement is not true of very strong solutions of peptones, which, he says, are precipitated by heat and mineral acids. The statement has not, however, been confirmed by the very careful examination by Herth of albumin peptone, nor does it agree with the author's observations. It doubtless rests upon the fact that Adamkiewicz worked with impure peptones.

Non-precipitation by acetic acid and ferrocyanide of potassium.

The most characteristic difference, according to authors, between solutions of peptones and all other proteid bodies, is found in the difference of behaviour to acetic acid and potassium ferrocyanide. Every proteid body in solution, with the single exception of peptones, is precipitated if acetic acid be first added and then a solution of potassium ferrocyanide.

It must not be supposed however that even the peptones are wholly unacted upon by these reagents, for, however carefully they may have been purified, it is found that the addition of acetic acid and potassium ferrocyanide, though at first without effect in the solution, which remains perfectly clear, ultimately causes some opalescence (Kühne and Chittenden); this is, however, very different from the abundant precipitation occasioned by the same reagents with other proteids.

Neutral and basic lead acetates only produce turbidity when added to solutions of peptones. Solution of copper sulphate (5 p.c.) does not affect a solution of ampho-peptones. Similarly, according to Kühne, solutions of platinum chloride, chromic acid, and ferric chloride are without action upon them.

Reagents which precipitate peptones. Peptones are precipitated by the following reagents:—tannic acid, mercuric chloride, mercuric and mercurous nitrates, Millon's reagent, potassio-mercuric iodide, and especially in feebly acid solutions by phospho-molybdic acid and phospho-tungstic acid. These two reagents furnish us with the means of separating peptones and have of late been much employed in research.

Preparation of phospho-tungstic acid. This reagent, which is of special value in the search for and separation of the peptones, is prepared as follows. Commercial tungstate of soda is dissolved in boiling water and phosphoric acid added to the solution until the mixture exhibits an acid reaction. It is then allowed to cool, rendered strongly acid with hydrochloric acid, and filtered after standing for twenty-four hours (Huppert)[1].

Preparation of phospho-molybdic acid. This reagent is made by digesting moist molybdic trioxide with solution of phosphoric acid. With a small quantity of the acid a lemon-yellow salt insoluble in water is formed. With a larger proportion of acid, the yellow salt dissolves on the application of heat, yielding a colourless liquid which, on evaporation, leaves a tenacious, non-crystalline, mass, very soluble in water and alcohol.

The action of Millon's reagent serves, as Kühne and Chittenden have shewn, to distinguish ampho-peptones from antipeptone (as obtained by the action of trypsin upon fibrin). When added to ampho-peptones the reagent at first throws down an abundant white precipitate which, on being warmed, assumes a beautiful red colour. With antipeptone, it gives a white precipitate, changing on heating to a dirty yellow or reddish colour. It is therefore obvious, that the source of the red reaction with ampho-peptone is to be sought for in the hemipeptone which forms a moiety of the ampho-peptone. To this matter, reference will again be made.

Colour reactions of the peptones. 1. Solutions of peptones exhibit when boiled with Millon's solution the characteristic proteid reaction.

2. Their most marked reaction is the so-called *biuret reaction*[2]. It is obtained by adding to a solution of peptone, a drop or two of a weak solution of copper sulphate, and then a large excess of sodium or potassium hydrate, when a fine, more or less deep, rose colour is obtained, with no violet tint. If the quantity of copper added is in excess, then a violet colour is produced.

As has already been stated, the biuret reaction is common to albumoses as well as peptones, though not given with equal intensity by all.

Peptones also exhibit the other colour reactions of solutions of the proteids, viz. the xantho-proteic reaction and the so-called 'Adamkiewicz's reaction.'

Adamkiewicz's Reaction. Any solution of a proteid or of a peptone when treated first of all with glacial acetic acid and then with concentrated sulphuric acid, acquires a beautiful violet colour, becomes weakly fluorescent, and exhibits in solutions, of suitable concentration, an absorption spectrum characterized by a band, similar to that of Urobilin, situated between Frauenhofer's lines *b* and F.

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[1] Quoted by v. Jacksch, 'Clinical Diagnosis,' p. 219.

[2] So-called because it is also obtained in a very characteristic manner with biuret, $C_2H_5N_3O_2$, a body obtained by the action of heat on urea.

Taste of the peptones.

While the native albumins and true albumins are free from any definite taste, peptones, for the most part, are characterised by an intensely bitter taste, which is particularly obvious in milk which has been subjected to the action of proteolytic ferments, even for a short time. It is most probable that, as Kühne suggested, the bitter taste does not belong to the peptones themselves but to bye products accompanying them, a surmise which is almost proved by the fact that peptones may be obtained which are free from the peculiar bitter taste, and that a secret method exists employed on a manufacturing scale (communicated confidentially to the author) whereby the bitter taste of gastric peptones may be readily destroyed without apparently otherwise affecting them in an appreciable manner.

Ampho-peptones have no action on the coagulation of the Blood.

It was shewn by the observations of Schmidt-Mülheim[1] and of Fano[2] that when a solution of de Witte's or Grübler's commercial peptones was injected into the blood-vessels of dogs, in the proportion of 0·3 grams of the commercial peptone per kilo of the body weight of the animal experimented upon, the blood lost its power of coagulating for a period of some hours.

Pollitzer[3], working under Kühne's direction, shewed however that neither pure ampho-peptones, nor antipeptone resulting from digestion with trypsin, possessed any decided power of influencing the coagulation of the blood, such action being due to bodies belonging to the albumose class, which were present in large quantities together with ampho-peptone in the commercial peptones of Witte employed by Schmidt-Mülheim and by Fano.

Cleavage of Ampho-peptone by the action of trypsin, as well as by persistent boiling with dilute sulphuric acid.

It has been stated, that ampho-peptone gives with Millon's reagent a very brilliant red colour, whilst antipeptone, when boiled with the same reagent, furnishes a yellow precipitate, at most tinged with red. Since Millon's reaction for proteids corresponds with Hoffmann's test for tyrosin, and since its production with proteids probably depends upon the formation of tyrosin, Kühne and Chittenden surmised that the cleavage products of ampho-peptone might be different from those of antipeptone. In accordance with this surmise, they found that a few hours' digestion, in a small tube, of 1 gramme of ampho-peptone in 10 c.c. of water containing 0·25 per cent. of Na_2CO_3 with a little thymol and and a fragment of purified trypsin, were sufficient to effect the cleavage of the peptone. On concentrating the neutralised solution, and boiling the residue with alcohol, a residue was obtained, in which, without further preparation, balls of leucin and bundles of crystals of tyrosin were to be seen under the microscope. From the products of such digestion, it was easy to separate tyrosin in a state of purity. Further, a few grams of the same preparation were heated for several days with six times their weight of sulphuric acid (2 parts acid and 3 of water) and after removing the acid with barium hydrate, tyrosin and leucin were found among the decomposition products.

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[1] Schmidt-Mülheim, 'Zur Kenntniss des Peptons und seiner physiolog. Bedeutung.' *Archiv f. Anat. u. Phys.* Phys. Abth. 1880, § 33—56.

[2] Fano, 'Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe.' *Archiv f. Anat. u. Phys.* Phys. Abth. 1881, § 277—297.

[3] Pollitzer, S., 'On the Physiological Action of Peptones and Albumoses.' *Journ. of Physiology*, Vol. VII. p. 283.

From antipeptone, subjected to the action of acid, on the other hand, whilst leucin was constantly, tyrosin was only occasionally obtained, and, then, in such traces as to be referable to accidental impurity of the antipeptone used.

Preparation of pure Hemipeptone and Antipeptone.

It is impossible, by any process as yet known to us, to prepare from ampho-peptone, or from its antecedents, a perfectly pure hemipeptone, uncontaminated by antipeptone. On the other hand, in consequence of the destructibility of hemipeptone on digestion with trypsin, antipeptone (*tryptone*) can be obtained from ampho-peptone uncontaminated by hemipeptone. A fuller description of antipeptone will be given under the pancreatic enzymes.

Diffusibility of the peptones.

Funke[1] pointed out that peptones in aqueous solution pass through filter paper, and diffuse through animal membranes and through parchment-paper, with much greater facility than other proteids. The considerable diffusibility through thin animal membranes is undoubted, and contrasts with that of other proteids; through good parchment-paper, though the diffusion of peptones is much greater than that of other proteids, it is absolutely very small, so that dialysis is employed with great advantage in freeing solutions of peptones from salts, but not to separate peptones from other colloidal bodies, as, for instance, from pepsin. The very sparing diffusibility of peptones through parchment-paper was first drawn attention to by v. Wittich[2], and afterwards confirmed by Maly, Herth, and many others.

Chemical Composition of the Peptones.

The researches of Maly[3], Herth[4] and Henninger[5], established that peptones do not, on ultimate organic analysis, differ in composition from the proteids which have yielded them, any differences which exist in chemical composition falling within the limits of experimental error.

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[1] Funke, *Lehrbuch d. Physiol.*, Vol. I. 5 Aufl. p. 208.

[2] v. Wittich, 'Ueber die Diffusibilität der Peptone.' *Berl. klin. Wochenschrift*, 1872, N. 37. Abstracted in Maly's *Jahresbericht*, Vol. II. p. 19. See also Hermann's *Handbuch*, Bd. V. 2. Th. S. 296.

[3] Maly, 'Ueber die chemische Zusammensetzung und physiologische Bedeutung der Peptone.' Pflüger's *Archiv*, Vol. IX. (1874), p. 585.

[4] Herth, 'Ueber die chemische Natur des Peptons und sein Verhältniss zum Eiweiss.' (Aus dem Lab. v. Prof. Maly.) Hoppe-Seyler's *Zeitschrift*, Vol. I. (1877), p. 277.

[5] Henninger, *De la nature et du rôle physiologique des peptones*. Paris, 1878.

The following table exhibits the results of ultimate organic analyses of fibrin and fibrin peptone, and of egg-albumin and egg-albumin peptone.

	Fibrin.	Fibrin-peptone.		Albumin.	Albumin-peptone.	
		1.	2.		1.	2.
	Maly.	Maly.	Henninger.	Herth.	Herth.	Henninger.
C	52·51	51·40	51·4	52·9	52·5	52·3
H	6·98	6·95	7·0	7·2	7·0	7·0
N	17·34	17·13	16·7	15·8	16·7	16·4
S				1·14	1·14	

The observations of Maly and of Herth seem also to shew that the peptone produced during the digestion of fibrin or of albumin is, in so far as ultimate analysis can determine the matter, one body; analyses of successively precipitated fractions giving identical results.

It must, however, be borne in mind that, in the case of bodies of so large molecular weight as the proteids and the peptones, identity in the results of their analysis is not sufficient to prove absolute identity in composition.

Divergent results obtained by Kühne and Chittenden.

Kühne and Chittenden have made a large number of analyses of various samples of ampho-peptone, as well as of antipeptone, but the results were marked by a remarkable and suspicious departure from uniformity. So great are the discrepancies that it is impossible to conceive that the substances designated by the same name were the same.

It appears to the author that in their attempts to purify and completely dry the peptones, these eminent investigators, adopted methods which, in all probability, profoundly modified the unstable substances subjected to them. The decomposition of the barium and phospho-tungstic acid compounds with sulphuric acid, and the heroic methods employed to dry the bodies submitted to analysis, could scarcely be expected ultimately to furnish products of which the analyses would be concordant among themselves, or which would exhibit any definite relationship with the mother substances from which they had been prepared.

Subjoined are the means of a large number of analyses of three samples of ampho-peptone, A, B, b, as well as the means of many analyses of antipeptones.

The following table exhibits the percentage composition of the various samples of peptones, calculated on the ash-free substance. The ash found, consisted in every case of calcium, a little sodium, potassium, traces of barium and iron, carbonic acid, phosphoric acid and sulphuric acid, and its amount is placed at the foot of the columns.

TABLE EXHIBITING RESULTS OF ANALYSES OF PEPTONES (KÜHNE AND CHITTENDEN).

	Ampho- (pepsin) peptone from fibrin.			Anti- (trypsin) peptone.					
	A.	B.	b.	C.	D.	E.	F.	G.	H.
				Prepared from fibrin.			Glandpeptone.		
	Containing mucin-peptone.	From purified pepsin and purified with phospho-tungstic acid.			Purified more with ether.	Purified with phospho-tungstic acid.		Purified with phospho-tungstic acid.	
C	44·53	48·75	48·47	47·30	47·68	46·59	44·45	42·96	44·47
H	6·49	7·21	7·02	6·73	7·03	6·69	7·17	7·26	7·15
N	16·73	16·26	16·86	16·83	16·68	18·28	17·06	17·80	17·94
S	0·72	0·77	...	0·73	...	0·67	0·50	0·31	0·57
O	31·53	27·01	...	28·41	...	27·77	30·82	31·67	29·87
Ash	8·11	3·22	2·15	5·25	10·02	3·67	5·54	1·93	2·07

The Relations of Peptones to the Proteids from which they are derived.

The facts, firstly, that peptones have approximately the same composition as the proteids which give rise to them; secondly, that peptones are reconverted in the organism with the greatest readiness into other albuminous bodies, render it well-nigh certain that the change which the proteid molecule undergoes in passing from the condition of a native albumin to that of a peptone is but a very slight one. It has been surmised (Hoppe-Seyler[1]) that peptones are hydration products of their respective proteids, which are their anhydrides. This view is borne out by the fact that peptones may be produced not only by the action of the enzymes of the alimentary canal (which by analogy with the action of ptyalin and diastase on starch are undoubtedly hydrolytic), but by other hydrolytic agencies, as by the action of superheated water, or steam, and by the prolonged action of dilute acids and alkalies, and of putrefaction. In this case, as in many others, the hydration product is characterized by a greater facility of entering into combination with acids and bases.

The view that peptones are products of hydration is supported by the two following sets of experimental facts.

1. Henninger heated 10 parts of fibrin-peptone with 25 parts of acetic anhydride acid for an hour; the acid was distilled off and the residue was treated with hot water, which dissolved a great part. The watery solution was dialysed, when there remained in the dialyser, a solution which coagulated on boiling, on the addition of nitric acid, and when treated with potassium ferrocyanide.

2. Henninger and Hofmeister[2] have by merely heating peptone to 140° obtained a body which when dissolved in water possessed the characters of a native albumin rather than of a peptone.

Whether the change which an albuminous body undergoes in being converted into a peptone is accompanied by hydration or not, there can be little if any doubt that it is a change in which a complex molecule is broken up into smaller molecules, and this surmise is supported by the greater diffusibility of peptones as compared with proteids.

THE ACTION OF THE GASTRIC JUICE UPON THE SO-CALLED "ALBUMINOID" BODIES.

There is a group of bodies, which have been described in connexion with the chemistry of the tissues of the animal body, which have a close genetic relationship to the albuminous bodies proper, and which are, therefore, often referred to as *albuminoid bodies;* these are collagen and gelatin, chondrin (?), mucin, elastin, keratin. We have now to examine the action of peptic digestion upon these bodies.

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[1] *Physiologische Chemie*, p. 227.

[2] *Zeitschrift f. physiol. Chem.*, Bd. II. (1878—79) S. 206. See also Pekelhäring, Pflüger's *Arch.*, Bd. XXII. (1880), S. 185, and XXVI. (1881), S. 515; Griessmayer. See Maly's *Ber.*, XIV. (1884), S. 26.

1. *Digestion of Collagen and Gelatin.*

By the action of pepsin and dilute hydrochloric acid, collagen (as in connective tissue and in bone) is converted into gelatin more rapidly than is the case when it is merely subjected to the action of dilute acids. Gelatin is acted upon by pepsin and hydrochloric acid. It slowly loses the property of separating from its solutions in the form of a jelly, and its specific laevo-rotatory power slightly diminishes[1].

According to Etzinger[2] the artificial digestion must be continued for 48 hours in order to deprive gelatin of the property of gelatinizing.

Uffelmann[3], however, observed that in the stomach of a boy with gastric fistula gelatin was so altered in the course of an hour as to be deprived of the power of gelatinizing, whilst he found that with artificial gastric juice digestion had to be continued for from 18 to 24 hours to produce the same effect.

As the ultimate product of the action of pepsin and hydrochloric acid on gelatin, there is produced a gelatin-peptone, or perhaps gelatin-peptones, which have yet to be subjected to thorough investigation. According to Tatarinoff, gelatin-peptone has an acid reaction, decomposes carbonates, and forms compounds with the alkaline earths, which have an alkaline reaction. Its solutions are said not to differ materially in their chemical reactions from solutions of gelatin[4].

2. *Digestion of Chondrigen and Chondrin*[5].

Chondrigen and chondrin are dissolved and digested somewhat more slowly than collagen and gelatin, with the formation first of acid-albumin, and then of peptone, in addition to a body which reduces cupric oxide in alkaline solution[6].

[1] J. de Bary, 'Untersuchungen über Leimstoffe.' Hoppe-Seyler's *Untersuchungen*, Heft i. (1866), p. 75.

[2] 'Etzinger, 'Ueber die Verdaulichkeit der leimgebenden Gewebe.' *Zeitschr. f. Biol.*, Vol. x. p. 84.

[3] Uffelmann, 'Untersuchungen an einem gastrotomirten fiebernden Knaben.' *Archiv f. klin. Med.*, Vol. xx. p. 535.

[4] Tatarinoff, 'Zur Kenntniss der Glutinverdauung.' *Centralblatt f. d. med. Wissenschaft* (1877, No. 16). Maly's *Jahresbericht*, Vol. VII. p. 277.

[5] The information given under this head is obtained entirely from Hoppe-Seyler, *Physiolog. Chemie*, p. 234.

[6] See Vol. I. p. 270, 'Products of Decomposition of Chondrin.'

3. *Digestion of Mucin.*

We possess few observations on the digestion of Mucin.

By prolonged boiling of a feebly alkaline solution of mucin, Eichwald obtained a body which he denominated *mucin-peptone*, which possessed essentially the characters of the peptones of the albuminous substances proper.

According to Kühne and Schiff it is not attacked by the constituents of the gastric juice. It would nevertheless appear probable that by the prolonged action of pepsin and hydrochloric acid, mucin undergoes some decomposition. The subject requires investigating anew.

4. *Digestion of Elastin.*

Etzinger[1] shewed that elastin when subjected to digestion for several days with pepsin and hydrochloric acid is dissolved. The Author finds that the solution of elastin, purified as perfectly as possible, is complete.

Horbaczewski[2] and Morochowetz[3] studied the products obtained by the action of artificial gastric juice upon elastin, the former observer extending his observations by studying the action of human gastric juice, the product of a gastric fistula, upon that substance.

Horbaczewski described two products of the action of artificial gastric juice upon elastin, to which he gave the name of hemi-elastin and elastin-peptone.

Chittenden and Hart[4] have made a still more elaborate investigation which has led them to the conclusion that, under the influence of pepsin and hydrochloric acid, there are formed two bodies, both belonging to the class of albumoses, and not to that of true peptones, inasmuch as they are precipitable by ammonium sulphate. To the first of these bodies, which corresponds to Horbaczewski's hemi-elastin, they give the name proto-elastose; this body is precipitated by NaCl. To the second, corresponding to Horbaczewski's elastin-peptone, they assign the name deutero-elastose. Both these bodies exhibit the biuret reaction.

The ultimate analyses of proto-elastose do not differ materially from those of the elastin which yielded it. On the other hand, the results of the analyses of elastin and deutero-elastose are not so concordant. These analyses do not, however, throw any special light upon the relations between the bodies.

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[1] Etzinger, *loc. cit.*

[2] Horbaczewski, 'Ueber das Verhalten des Elastins bei der Pepsinverdauung,' *Zeitschrift f. physiol. Chemie*, Vol. VI. p. 330.

[3] Morochowetz, 'Verdauungsgesetze.' Maly's *Jahresbericht* (1886), p. 271.

[4] R. H. Chittenden and A. S. Hart, 'Elastin und Elastosen.' *Zeitschrift f. Biolog.*, Vol. XXV. (1889), p. 368.

5. *Action of Pepsin and Hydrochloric Acid on Keratin and Chitin.*

The keratin- and chitin-yielding structures are not attacked by pepsin and hydrochloric acid.

6. *Action of Pepsin and Hydrochloric Acid on Oxy-haemoglobin.*

Oxy-haemoglobin is rapidly decomposed by natural or artificial gastric juice, with the formation, on the one hand, of albumoses and peptone, and on the other of haematin which undergoes no further decomposition[1].

SECT. 11. THE MILK-CURDLING ENZYME (OR RENNET-ENZYME) OF THE STOMACH.

(Syn. *Rennin*, Lea, Foster. *Chymosin*, Deschamps.)

It has long been known that the mucous membrane of the fourth or true stomach of the calf possesses the property of curdling milk, and various preparations of this mucous membrane have, under the name of 'rennet,' been employed to coagulate casein in the manufacture of cheese. It has also long been known that the gastric juice, when added to milk, curdles it; this action has been ascribed, by some, to the pepsin, and, by others, with greater justice, to the free acid which it contains.

It was, however, shewn, first of all by Heintz[2], that the mucous membrane of the stomach possesses the property of curdling milk when the reaction is neutral, and even alkaline. The recent researches of Hammarsten[3] have demonstrated that the milk-curdling property depends upon the presence of an enzyme of which the zymogen is present in the gastric mucous membrane.

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[1] Hoppe-Seyler, *Physiolog. Chemie*, p. 233.

[2] W. Heintz, 'Ueber die Ursache der Coagulation des Milchcaseins durch Lab und über die sogenannte amphotere Reaction.' *Journ. f. prakt. Chemie*, Neue Folge, Vol. VI. p. 374.

[3] Hammarsten, 'Ueber die Milchgerinnung und die dabei wirkenden Fermente der Magenschleimhaut.' Maly's *Jahresbericht*, Vol. II. (1872), p. 118. 'Ueber den chemischen Verlauf bei der Gerinnung des Caseins mit Lab.' Maly's *Jahresbericht*, Vol. IV. (1874), p. 135. 'Zur Kenntniss des Caseins und der Wirkung des Labferments,' Upsala, 1877, and Maly's *Jahresbericht*, Vol. VII. (1877), p. 158. The articles in Maly's *Jahresbericht* are by Hammarsten himself, and give a very full account of the original Swedish papers. The third paper was published *in extenso* in German.

The milk-curdling ferment a constant ingredient of Human Gastric Juice.

One of the most interesting facts connected with the composition of the gastric juice of man, and which deserves to be brought into much greater prominence than has been usual, is that in the adult human subject in a state of health the rennet ferment is never absent in the physiological condition. It is only in cancer, in atrophy of the mucous membrane of the stomach, and in chronic gastric catarrh, that it has been found absent.

The milk-curdling enzyme or its zymogen reside in the gastric mucous membrane of certain animals.

The mucous membrane of the stomach of the calf and of the sheep always contains *ready-formed* milk-curdling ferment, which can be extracted from it by the action of water and other solvents to be mentioned hereafter; most frequently none can be extracted by water from the mucous membrane of the stomach of other mammals or of birds, and it is scarcely ever present in that of fishes.

Although the free ferment removeable by water is rarely found, Hammarsten has shewn *that the gastric mucous membrane of all animals in which it has been investigated, without exception, contains a body which is not the milk-curdling ferment, but its zymogen: from this the milk-curdling ferment is quickly liberated on the addition of an acid.*

Distribution of the milk-curdling enzyme in the mucous membrane of the stomach.

Hammarsten has found that the mucous membrane of the fundus is very much richer in the milk-curdling ferment and its zymogen than that of the pylorus. So far as experiments have been made, variations in the amount of rennet ferment (including rennet-zymogen), in the gastric mucous membrane, run parallel with variations in the amount of pepsin (including pepsinogen). Probably rennet-zymogen, like pepsinogen, takes its origin in the granules of the chief cells.

Mode of preparing an active solution of the ferment and of observing its action.

Although the mucous membrane of the stomach of the calf and of the sheep always yields to water having a neutral reaction a sufficient quantity of milk-curdling ferment to demonstrate its peculiar properties, much more powerfully-acting solutions are obtained by the aid of dilute acids, as follows:—The mucous membrane of the stomach, preferably of a calf, is digested for 24 hours, at ordinary temperatures, in 150—200 c.c. of very dilute hydrochloric acid, containing from 0·1 to 0·2 per cent. of HCl. The liquid is then filtered and carefully neutralized. Twenty-five c.c. of fresh milk are then heated to 38° C., and treated with 1 c.c. of the neutralized liquid. Curdling is induced within a period of two minutes; this occurs even if the milk have been rendered faintly alkaline by caustic soda; the alkaline reaction persists after curdling. A glycerin-extract of the stomach of the calf may be used, as Hammarsten first shewed, instead of the solution prepared as stated above; such a glycerin-extract can be preserved permanently, and is very active. Erlenmeyer[1] has shewn that a saturated aqueous solution of salicylic acid extracts the milk-curdling ferment, very perfectly, from the stomach of the calf; from this solution, the ferment, mixed with other matters, can be precipitated by alcohol. The precipitate thus obtained is soluble, in great part, in water, and the solution is active.

The milk-curdling ferment separated from pepsin.

Hammarsten separated the ferment free from pepsin by the fractional precipitation of a solution containing both enzymes, with lead acetate. He first prepared a HCl infusion of the gastric mucous membrane by the aid of dilute HCl, and this infusion he neutralised by repeatedly shaking it with magnesium carbonate; he then added, to the neutralised solution, enough lead acetate to precipitate the whole of the pepsin as completely as possible, so that when the filtrate was acidulated and digested for 24 hours with a flake of fibrin it was not perceptibly dissolved. To the liquid from which pepsin had been precipitated there was added more acetate of lead and a little solution of ammonia. The precipitate thus obtained was decomposed with highly diluted sulphuric acid. Thus the milk-curdling ferment which had been precipitated by the second addition of lead acetate was set free. From the acid solution, the ferment was obtained, by a modification of the cholesterin-process employed by Brücke for the separation of pepsin.

Characters of solutions of the pure (?) milk-curdling enzyme.

A solution of the pure milk-curdling ferment, prepared as described above, possesses the following reactions:—it does not give the xantho-proteic reaction; it is not coagulated by heat; it is not precipitated by alcohol, nitric acid, tannin, iodine, nor by sugar of lead, but by basic lead acetate.

The milk-curdling ferment is not diffusible; it does not pass through porous earthenware unless the pressure be very high.

Various agents which diminish, or destroy, the action of the milk-curdling ferment.

1. Alcohol slowly destroys the activity of the ferment, the rapidity of the action being influenced by the quantity of alcohol.

2. Fixed caustic alkalies have a powerfully destructive action. If a solution rich in ferment contains as much caustic soda as corresponds to 0·025 per cent. of Na_2O, it loses its activity entirely within 24 hours at a temperature of 15—17° C.

3. Rennet ferment is also destroyed by 0·5 to 1 per cent. of Na_2CO_3[2]. Since rennet-zymogen is much less readily destroyed,

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[1] Erlenmeyer, 'Darstellung der ungeformten Fermente.' Communicated to the Acad. of Sciences of Munich in 1875 and abstracted in Maly's *Jahresbericht*, Vol. v. p. 267.

[2] Langley, *Journal of Physiology*, Vol. III. (1882), p. 259.

dilute sodium carbonate may be used—as in the case of pepsinogen and pepsin—to distinguish zymogen from ferment[1].

4. High temperatures diminish, and ultimately destroy, the activity of solutions of the curdling ferment, the result being remarkably influenced, however, by the reaction of the liquid. A liquid which is very rich in ferment can be momentarily heated to 70° C. without losing all its activity, whilst if the same liquid be acidulated with 0·3 per cent. of HCl, momentary heating to 63° C. or digestion at 37°—40° C. for 40 hours, suffice completely to annul its milk-curdling power. As pepsin is not destroyed by long digestion in acid solutions, whilst the milk-curdling ferment is so destroyed, we can free *acid* pepsin solutions from the curdling ferment by merely keeping them for a sufficient time at 40° C.

The one characteristic property of the ferment is to coagulate casein.

The one great characteristic of the rennet ferment is the power which it possesses of clotting solutions of casein, providing these contain a sufficient quantity of lime salts.

Without anticipating the complete treatment of this subject in another volume of this work, it is necessary in this place to state that comparatively recent researches of Hammarsten have established that casein, as it exists in milk, is a body which can be precipitated unaltered, by means of various agents, such as common salt, magnesium sulphate and very weak acids. Under the influence of rennet, however, casein probably undergoes a decomposition which appears to resemble that of fibrinogen, as we conceive it to occur when under the influence of the fibrin-ferment, it splits up with the separation of fibrin. Casein under the influence of the ferment would appear to split up into a proteid, which if lime salts be present, assumes a clotted form, and into one which remains in solution.

Paracasein and whey-albumin (whey-albumose).

To the solid product formed during the clotting of milk or of a solution of casein by milk, we may with propriety apply the name *Paracasein*, especially as this appropriate term is the suggestion of Hammarsten, to whose researches we are indebted for our present knowledge of the clotting of casein. The soluble product of the decomposition is a proteid, resembling the albumoses in its reactions, to which Hammarsten gives the name of Molkeneiweiss, or whey-albumin (why not Molken-albumose or whey-albumose ?).

The milk-curdling ferment does not convert milk-sugar into lactic acid.

Activity of the milk-curdling enzyme.

Hammarsten precipitated a glycerin-extract of calf's stomach with alcohol, and dissolved the precipitate in water. The amount of dissolved matter was then determined, and also its power of coagulating casein. Assuming all the dissolved substance to consist of pure ferment, it would curdle from 400,000 to 800,000 times its weight of casein.

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[1] Langley, *Journal of Physiology*, Vol. III. (1882), p. 287.

The relative activity of solutions of milk-curdling ferment of unknown strength is determined by observing, *caeteris paribus*, the relative rapidity with which they induce the curdling of milk.

The nature of the process which goes on in the curdling of milk under the influence of the milk-curdling ferment will be treated of at length under Milk.

SECT. 12. ASSUMED EXISTENCE OF A LACTIC ACID FERMENT IN THE STOMACH.

Hammarsten made the observation that pure solutions of the milk-curdling ferment, just as pure solutions of pepsin, exert no action on milk-sugar or on proteid solutions containing milk-sugar, whilst gastric mucus or the neutralized infusion of stomach possess the property of engendering the lactic acid fermentation. Pepsin and milk-curdling ferment can be destroyed by the action of dilute solutions of caustic soda, and the liquid thus freed from these two ferments still possesses the power of converting milk-sugar into lactic acid. Hammarsten thence conceives that there exists in the mucous membrane of the stomach a third lactic-acid-forming ferment. It appears to the author that before assuming the presence of an unformed ferment exerting this action, the presence of lactic-acid-generating micro-organisms would have to be more particularly disproved.

SECT. 13. THE PROCESS OF DIGESTION IN THE LIVING STOMACH.

Having now studied the chemical composition of the gastric juice, the character of its separate constituents, and the action which they exert upon the particular class of proximate principles which are acted upon in the stomach, it remains to consider the actual process of digestion as it occurs in the living organ, and in doing so we shall be brought face to face with certain questions which have not been discussed in the preceding sections. Although experiments on artificial digestion teach us the nature of the process which occurs in the stomach, we cannot pretend that such experiments will furnish us with data which will apply exactly to the stomach, for in this organ we have conditions which are very different from those which exist *in vitro*. In the stomach, we have no ordinary receptacle, into which artificial gastric juice is poured, so as to be mixed with food, but a receptacle kept constantly at a temperature most favourable to digestion, provided with arrangements which enable it continually to alter its capacity according to the mass which it contains, which it grasps, and kneads and rotates—a perfectly contrived arrangement for continually mixing the food to be dissolved with the solvent juice. The stomach is a receptacle, too, in which absorption is continually going on, of water holding certain substances in solution, and secretion of the pepsin and acid needed to carry on the digestive process; a receptacle from which at a certain period of digestion, the more finely subdivided matter is gradually drained away, through the pyloric orifice, leaving the grosser masses to be further subjected to the combined influence of mechanical movements and the solvent action of gastric juice.

General Sketch of Digestion in the Living Stomach.

When food is introduced into the living stomach, the mucous membrane which was previously pallid becomes injected: droplets of liquid commence to exude from the open mouths of the gastric glands, and uniting, form a stream of gastric juice. At the same time the organ contracts around the mass which it contains, and complex movements occur which cause "not only a constant disturbance or churning of the contents of the organ, but compel them, at the same time, to revolve around the interior from point to point and from one extremity to the other[1]." In order to form a proper conception of the stomach during digestion we must not think of it as a flaccid sac of definite form, such as it appears after death, but as varying in size and also in shape as its walls grasp tightly the mass which it contains, and as waves of contraction slowly pass over it, forcing the mass round and round in various planes.

"When food first enters the stomach the movements are feeble and slight, but as digestion goes on they become more and more vigorous, giving rise to a sort of churning within the stomach, the food travelling from the cardiac orifice along the greater curvature to the pylorus, and returning by the lesser curvature, while at the same time subsidiary currents tend to carry the food which has been passing close to the mucous membrane towards the middle of the stomach. and vice versa[2]."

"While these revolutions of the contents of the stomach are progressing, the trituration or agitation is also going on. There is a perfect admixture of the whole ingesta, during the period of alimentation and chymification. There is nothing of the distinct lines of separation between old and new food, and peculiar central or peripheral situation of crude, as distinguished from chymified aliment, said to have been observed by Philip, Magendie, and others in their experiments on dogs and rabbits, to be seen in the human stomach; at least in that of the subject of these experiments. The whole contents of the stomach, until chymification be nearly complete, exhibit a heterogeneous mass of solids and fluids; hard and soft; coarse and fine; crude and chymified; all intimately mixed, and circulating promiscuously through the gastric cavity, like the mixed contents of a closed vessel, gently agitated or turned in the hand.".................."As the food becomes more and more changed from its crude to its chymified state, the acidity of the gastric fluids is considerably increased, and the general contractile force of the muscles of the stomach is augmented in every direction; giving the contained fluids an impulse towards the pylorus. It is probable that from the very commencement of chymification—from the time that food is received into the stomach until that organ becomes empty—portions of chyme are constantly passing into the duodenum through the pyloric orifice, as the mass is presented at each successive revolution. I infer this from the fact that the volume is constantly decreasing. This decrease of volume, however, is slow at first; but is rapidly accelerated towards the conclusion of digestion, when the whole mass becomes more or less chymified. This accelerated expulsion appears to be effected by a peculiar action of the transverse muscles, or rather of the *transverse band*......... situated near the commencement of the more conical shaped part of the pyloric extremity, three or four inches from the smaller end. In attempting to pass a long glass thermometer tube through the aperture into the pyloric portion of the stomach, during the latter stages of digestion, a forcible contraction is first perceived at this point, and the bulb is stopped. In a short time, there is a gentle relaxation, when the bulb passes without difficulty, and appears to be drawn, quite forcibly, for three or four inches, towards the pyloric end. It is then released, and forced back, or suffered to rise again; at the same time giving to the tube a circular, or rather spiral motion, and frequently revolving it completely over. These motions are distinctly indicated, and strongly felt, in holding the end of the tube between the thumb and finger; and it requires a pretty forcible grasp to prevent it from slipping from the hand, and being drawn suddenly down to the pyloric extremity. When the tube is left to its own direction, at these periods of contraction, it is drawn in, nearly its whole length, to the depth of ten inches: and when drawn back, requires considerable force, and gives to the fingers the sensation of a strong *suction-power*, like drawing the piston from an exhausted tube...............These peculiar motions and contractions continue until the stomach is perfectly empty and not a particle of the food or chyme remains; when all becomes quiescent again..........The peculiar contractions and relaxations, mentioned above, succeed each other, at regular intervals of from two to four or five minutes. Simultaneously with the contractions there is a general shortening of the fibres of the stomach. This organ contracts upon itself in every direction; and its contents are compressed with much force.. ....During the intervals of relaxation, the rugae perform their vermicular actions, the undulatory motions of the fluids continue, and the alimentary and chymous masses appear, revolving as before, promiscuously mixed, through the splenic and cardiac portions[1]."

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[1] Beaumont, *Experiments and Observations on the Gastric Juice.* Edinburgh Edition, 1838, p. 101.

[2] Foster, *Physiology*, 3rd ed. (1879), p. 270.

In quoting *verbatim* considerable portions of Dr Beaumont's vivid and unique observations on his patient, St Martin, the Author does so because the description gives some idea of the intensity of the mechanical movements which aid the chemical action of the gastric juice so efficiently as to enable the stomach to effect digestive operations which, in point of magnitude, cannot be imitated in the laboratory.

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[1] Beaumont, *Op. cit.*, p. 102 *et seq.*

The term Chyme explained.

The term Chyme (χυμός, juice) was formerly generally applied to the pulpy semi-fluid matter resulting from the action of the gastric juice on the mixed aliments, and the term Chymification to the process which results in the formation of chyme.

The stomach the seat of absorption of much water, and of diffusible substances.

Doubtless when much fluid is introduced into the stomach, absorption at once commences actively. This is proved by the fact (amongst others) that almost instantly the sensation of thirst, which depends primarily upon a diminution of the water of the blood, diminishes. At the same time, the absorption of some diffusible substances occurs, as is proved by the fact that a few minutes after the introduction of potassium iodide into the stomach, the salt is separated by the kidneys and salivary glands. The extent to which the process of absorption proceeds in the stomach cannot, however, be exactly stated. This subject will be more fully considered in the sequel.

See p. 439

There is an interesting observation, which has repeatedly been made in dogs with gastric fistulae, which proves both the rapidity with which absorption goes on and the fact that thirst, though a local sensation, is yet but the expression of the general want of the system for water. If in a dog with a gastric fistula, the cannula be allowed to remain unplugged until the animal manifests decided thirst, then on water being given to it, the animal will commence drinking and will continue to drink indefinitely, the water running out through the cannula as fast as it enters the stomach. So soon however as the cannula is plugged, the animal behaves as normal animals do, and, after a very small quantity of fluid has been taken, stops drinking; in a small fraction of a minute sufficient absorption of water has occurred to relieve the general want for water, of which the sensation of thirst was the local expression.

The constituents of food which are acted upon chemically in the stomach.

The constituents which are chemically acted upon by the gastric juice in the stomach are firstly the proteids, and secondly the *albuminoid* bodies, collagen and gelatin, chondrigen and chondrin (?).

The other groups of organic food constituents, viz. fats and carbohydrates, are very slightly acted upon by the gastric juice itself; in considering this slight action of the gastric juice we shall have to enquire to what extent the amylolytic action of the saliva upon the alimentary starch is allowed to proceed in the presence of the acid juices of the stomach.

The Changes which Adipose Tissue undergoes in the Stomach.

Until lately the majority of authorities have held that the fatty constituents of the food undergo no change in the stomach, although their subsequent digestion in the small intestine is promoted by the solution of the walls of the fat cells which occurs in the stomach, and which liberates their fatty contents.

It was stated by Dr Marcet, however, that a certain decomposition of the neutral fats takes place in the stomach. In a recent research Cash[1] has found that when dogs are fed upon perfectly neutral fats, fatty acids are liberated in small quantities, and that when the mucous membrane of the stomach is digested with neutral fats, in the presence of dilute hydrochloric acid, fatty acids are likewise liberated. The setting free of traces of fatty acids, if it occurs, will aid the subsequent emulsionizing of the fats by the bile and pancreatic juice.

The Changes which Starch undergoes in the Stomach.

In discussing the changes which starch undergoes in the stomach, we have to consider, firstly whether the gastric juice possesses by itself any action upon starch, and secondly to what extent the action of the saliva upon starch continues in the stomach.

Does the gastric juice alone possess any action upon starch?

The saliva of many animals, e.g. the dog, is devoid of diastatic properties. If a dog be fed upon a meal of boiled starch and killed during digestion whilst the stomach still contains food, mere traces of sugar are found, but the contents contain both soluble starch and erythrodextrin (Brücke[2]). Unboiled starch is unacted upon.

Does the action of saliva upon the starchy constituents of food continue in the stomach?

The contents of the stomach of man fed upon a diet containing boiled starch always contain considerable quantities of sugar, and the question arises, Were these produced by the momentary action of saliva upon starch during mastication and deglutition, or did the conversion of sugar under the influence of saliva continue in the stomach? In endeavouring to solve this question, we have to bear in mind, firstly, that diastatic ferments do exert their action upon starch in a fluid of feebly acid reaction, but secondly, that that action is arrested so soon as the reaction becomes strongly acid. It would therefore appear most likely that in the early stages of gastric digestion, before the admixture with gastric juice is complete and when the acidity of the gastric juice is comparatively feeble, the diastatic action of the saliva proceeds in the stomach, whereas soon after, when the acid reaction has attained a certain figure, diastatic action diminishes or even ceases altogether.

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[1] Cash, *Archiv f. Anat. u. Physiologie*, 1880. *Physiol. Abtheil.*, p. 323.

[2] Brücke, *Sitzungsber. d. Wiener Acad.* 3 Abth. Vol. LXV. (1872.)

The statements of various authors concerning the influence of an acid reaction upon the amylolytic action of the salivary ferment differ remarkably. Thus Brücke asserts that in a solution containing 0·5 of HCl per 1000, the conversion of starch into sugar goes on, whilst when the quantity reaches 1 per 1000, no action on starch occurs[1]. Hammarsten found that the diastatic action ceased when the quantity[2] of hydrochloric acid amounted to from 0·05—0·25 per cent. Langley[3] observed that when saliva is digested with HCl of from 0·2 to 0·04 per cent. for times varying from 24 to 7 hours the ferment was destroyed. On the other hand, Richet[4] asserts that saliva exerts a more powerful action on starch in the presence of 2 parts per 1000 of hydrochloric acid, than when the reaction is neutral or feebly alkaline, and Defresne contends that diastatic action goes on unimpeded by the gastric juice.

In forming an opinion concerning the important question as to the extent of starch digestion, and its duration in the stomach, we must rely chiefly on the researches of Langley and of Chittenden, although in some particulars these observers do not agree.

In 1881 Chittenden[5] announced that the ferment of saliva was destroyed on being warmed for two hours with gastric juice containing 0·2 per cent. of hydrochloric acid, and even that much smaller percentages of acid, as 0·025 per cent., diminished the diastatic action of the ferment very materially.

Langley[3] independently pointed out, in a study on the destruction of ferments in the alimentary canal, that weak solutions of the salivary ferment were destroyed by heating at 40° C. with 0·014 per cent. HCl.

Chittenden and Griswold[5], although finding that the salivary ferment was destroyed by very small quantities of free acid, arrived at the curious result that a smaller quantity of acid increased the diastatic activity. The explanation of this phenomenon was given by Langley, who found that neutralised saliva converts starch into sugar much more actively than unneutralised saliva.

When therefore starch mixed with saliva enters the stomach, the diastatic action will proceed, and up to a certain point may go on more rapidly than with saliva which has not been neutralised. As the acid reaction becomes strongly developed the action will, however, rapidly slow and be arrested.

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[1] Brücke, *Vorlesungen*, p. 280.

[2] Hammarsten, 'Einwirkung von Speichel auf Stärke.' Maly's *Jahresbericht*, Vol. I. p. 187.

[3] Langley, 'On the Destruction of Ferments in the Alimentary Canal.' *Journal of Physiology*, Vol. III. (1882), p. 246.

[4] Richet, 'Du Suc Gastrique.' *Journ. de l'Anat. et de la Physiologie*, Vol. XIV. (1878), p. 285.

[5] Chittenden and Griswold, *Amer. Chem. Journ.*, Vol. III. p. 303. See Chittenden and Smith, 'The diastatic action of Saliva, as modified by various conditions, studied quantitatively.' *Studies from the Lab. of Phys. Chem. of Yale College*, for the year 1884—85. New Haven, 1885, p. 1, *et seq.*

In connection with the neutralisation of saliva by acid we have to consider a point, concerning which there is a disagreement between Langley and Chittenden. It had been shewn by Danilewsky[1] that various proteids combine with acids and alkalies, or with one of them, and Langley[2] found that in the case of saliva, because of the proteids which it contains, a certain amount of acid may be added without there being any acid free, because of the formation of acid-proteid. Now, according to Langley, the acid-proteid which is formed when saliva is neutralised with acids acts prejudicially on the diastatic action of the salivary ferment, whilst according to Chittenden and Smith[3], the influence of 'acid-proteid matter' seems to stimulate the diastatic action, up to a certain point, larger quantities ultimately slowing, and even destroying, the ferment.

Summarising, then, the researches to which we have alluded we may safely conclude that in the first stage of gastric digestion, i.e. for a period up to about half-an-hour, the conversion of boiled starch into dextrins and maltose doubtless proceeds actively, but that it then ceases, under the influence of acid-proteids and free acid.

Is the salivary diastatic ferment destroyed in the stomach?

Closely connected with the question just discussed is one to which we have incidentally referred, viz. whether the diastatic ferment is destroyed or not in the stomach. Upon this matter, also, the earlier statements of authors differed very greatly. Thus it was said by Cohnheim[4] that the diastatic ferment is not destroyed when submitted to artificial digestion with pepsin and hydrochloric acid for many hours, for on neutralising the liquid it was found to possess diastatic powers. Schiff[5] made the same statement; and more recently Defresne[6] has repeated it. Roberts[7], on the other hand, asserted that the diastatic power of the saliva is quickly and permanently abolished both by an artificial digestive fluid and by filtered gastric juice obtained from the human stomach. On this subject we refer to the more recent experiments of Chittenden and of Langley, already referred to, as furnishing us with the most valuable and reliable information, and as proving conclusively the ultimate destruction of diastatic ferment in the stomach.

Changes in the Acidity of the Contents of the Stomach during Digestion.

It has been already said that the acidity of the contents of the stomach increases as digestion proceeds, and attention must now be directed to variations which occur simultaneously in the nature of the free acid.

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[1] Danilewsky, *Centralblatt f. d. med. Wissenschaft*, 1880, quoted by Langley.

[2] J. N. Langley and F. Eves, 'On certain conditions which influence the amylolytic action of saliva.' *Journal of Physiology*, Vol. IV. p. 18.

[3] Chittenden and Smith, *loc. cit.*

[4] Cohnheim, 'Zur Kenntniss der zuckerbildenden Fermente.' Virchow's *Archiv*, Vol. XXVIII. (1863), p. 248.

[5] Schiff, *Leçons sur la Digestion*, Vol. I. p. 162.

[6] Defresne, 'Études comparatifs sur la ptyaline et la diastase.' *Comptes Rendus*, Vol. LXXXIX. p. 1070.

[7] Roberts, *On the Digestive Ferments, &c.* London and Manchester, 1880, p. 53.

Richet's observations on the acidity of the contents of the stomach?

It has been shewn, in a previous section, that the acid reaction of the gastric juice is due to the presence of free hydrochloric acid, though Richet maintains, of hydrochloric acid in combination with an amido-acid, such as leucine. V. den Velden[1] asserts that in the first stages of digestion in the human stomach no free hydrochloric acid can be detected until three-quarters of an hour after a full meal such as dinner.

It must be remembered, however, that the proteids which are present in the gastric juice interfere very materially with the colour reactions upon which reliance is placed in asserting the presence or absence of free hydrochloric acid. We shall probably be near the truth, therefore, in asserting that at least twenty minutes or half-an-hour, and occasionally forty minutes, must elapse before the stomach contents contain an *appreciable quantity* of free hydrochloric acid.

The gastric juice behaves, it was shewn, when shaken with ether, as an aqueous solution containing a mineral acid.

The pure gastric juice of man has an acidity which, according to Richet's observations, corresponds to 1·3 parts by weight of HCl in 1000.

When digestion is proceeding, however, the acidity increases somewhat. However large the quantity of liquid in the stomach, it is found to have an acidity which on an average (according to Richet) corresponds to 1·7 parts of HCl per 1000, though it may, especially at the end of digestion, exceed this figure somewhat. After the ingestion of acids or of alkalies, the normal acidity is soon re-established.

Richet has found that the acidity of the contents of the stomach in the advanced stages of digestion no longer depends solely on a mineral acid, but that considerable quantities of acids soluble in ether are present. These acids are, in part, produced by the decomposition of salts of organic acids present in the ingested food, but, according to Richet, they are, in no small degree, due to acids which result from acid fermentations which occur in the stomach. Thus in the case of milk, according to Richet, there is set up, as a normal process, an acid fermentation which leads to the development of large quantities of lactic acid. The feebler the normal acidity of the gastric juice, the greater the quantity of the organic acids resulting from fermentative changes. It is probable that the acids thus set free reinforce the normal acid and cooperate in the process of digestion.

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[1] Von den Velden, 'Zur Lehre von der Wirkung des Mundspeichels im Magen.' *Zeitschrift f. phys. Chemie*, Vol. III. p. 205.

Duration of the Digestive Process in the Stomach.

The digestive process varies in duration in different animals, and in the same animal according to the nature of the food, to its state of division &c. In Alexis St Martin, Dr Beaumont found the duration of the gastric digestive process to be between three to five hours, and Richet remarks, as the result of his observations on his patient Marcelin, that the digestive process does not appear to extend beyond four or five hours, though three hours represent its more usual duration[1].

In dogs and other carnivorous animals which are in the habit of 'bolting' large masses of meat, undigested masses are occasionally found in the stomach eight or ten hours after a meal and often longer.

In connection with this question we have to consider the facts which relate to the manner in which the stomach empties itself.

According to some physiologists, almost from the earliest moments of gastric digestion, the patulous pyloric orifice allows the soft, already chymified portions of the contents, to escape into the duodenum, whilst the yet solid contents, not being able to escape, are mechanically retained and forced to revolve and revolve, until, under the influence of fresh juice, of the heat, of the mechanical movements of the compressing stomach, they themselves break down into, and form part, of the grumous chymified mass.

By a gradual process, then, the stomach, according to this view, gradually empties itself.

The author is not disposed to believe that matters proceed precisely in this manner, but rather as has been described by Richet and by Kühne.

According to Richet, whilst doubtless the softer and more diffluent portion of the gastric contents do, little by little, escape from the stomach into the duodenum, the quantity thus escaping is insignificant, the stomach contents remaining essentially undiminished during the average digestive period of three hours, at the conclusion of which, within a very short time, the whole of the contents are emptied into the duodenum.

The process, according to Kühne, is very similar in the dog, except that the average duration of gastric digestion is five, and not three, hours. During these five hours, at intervals of about ten minutes, the stomach expels small quantities of its contents through the pylorus; the great mass remains, however, to be expelled, almost at one time, when the act of gastric digestion comes to an end.

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[1] The reader is referred to a very interesting set of observations, on the duration of the digestive process in a thoroughly healthy man of 30, made by Jessen. The duration of the digestive process was judged of by the stomach-pump. 100 grammes of raw beef + 1 gramme of salt and 300 c.c. of water were digested in 2 hours. If the meat were thoroughly boiled or roasted (underdone) the time occupied in digestion was 3 hours.

602 c.c. of fresh unboiled cow's milk were completely digested in 3½ hours, but the same quantity of boiled cow's milk required 4 hours (*Zeitschrift fur Biologie*, Vol. XIX. (1883), p. 149).

The final Products of Digestion which leave the Stomach. The Chyme.

As a result of the combined influence of the gastric juice, of the movements of the stomach, and of the high temperature of the organ, the solid alimentary matters are reduced to a pulpy or semi-fluid condition, and it is in this state that they escape through the pylorus into the duodenum.

During the digestive process, large quantities of proteids and of albuminoid bodies have been converted into peptones, of which doubtless a part—though we have no data on the subject—is absorbed by the gastric mucous membrane as soon as formed, whilst a part is held in solution in the liquid portion of the *chyme*. As a result of the action of the acid of the gastric juice, insoluble mineral salts, as e.g. bone-earth, are dissolved and doubtless are absorbed, as are also soluble salts, sugar, and large quantities of water.

The chyme then must contain chiefly the undigested or partially digested fragments of food, mixed with gastric juice holding in solution products of digestion.

Accordingly we observe it to contain fragments of muscle, individual muscular fibres, splitting into fibrils, but especially tending to cleave into transverse discs. The fibrillar connective tissue has, wholly or in great part, disappeared, but yellow elastic tissue is found apparently quite intact; the same remark applies to cellulose and to the epidermal tissues of animals. If raw starch has been partaken of, the chyme is sure to contain unaltered starch grains.

Lastly, if adipose tissue or any fat was contained in the food, drops of liquid fat are found in the chyme. It has been observed by Richet that where the contents of the stomach contain much fat, this appears to be retained in the stomach until all other matters have been expelled.

SECT. 14. THE NON-DIGESTION OF THE STOMACH BY ITS JUICE.

The fact that the delicate mucous membrane of the living stomach is not digested by the gastric juice which it secretes early attracted the attention of observers.

Post-mortem digestion of the stomach.

John Hunter[1] was the first to draw attention to the fact that when animals or human beings are killed whilst the digestive process is actively proceeding, it not unfrequently happens that large portions of the stomach are softened and perforated. The gastric juice, then escaping, may act upon adjacent organs, partially digesting them, as in a case which came under the notice of the Author, and in which a part of the spleen had been pretty thoroughly digested, and the left half of the diaphragm had been perforated. The process proceeds most perfectly when the external conditions are such that the body cools slowly; it affects particularly the fundus of the stomach.

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[1] John Hunter 'Observations on Certain Parts of the Animal Economy,' London, 1786, and *Philosophical Transactions* for 1772.

Why is the living stomach not digested by its own juice?

John Hunter attempted to explain the non-solution of the living stomach by the gastric juice as due to its vital properties,—to the 'living principle'—, which exempted it from an action which dead matter could not resist. But this explanation, besides being open to the objection of a *petitio principii*, is disproved by the fact that living tissues may, under certain circumstances, be digested by the stomach. Thus Claude Bernard found that the legs of a living frog which had been introduced through a fistula into the interior of the stomach of a dog underwent digestion, though the animal was alive.

Claude Bernard explained the non-digestion of the gastric mucous membrane as due to its epithelial covering, which is continually being renewed, whilst Schiff believed that the layer of mucus which covers the internal surface of the stomach effectually protects it. The view of Claude Bernard is disproved by the fact that in cases where the continuity of the epithelial covering of the stomach is interrupted, as in gastric ulcer, digestion of the parts deprived of epithelium does not occur. Schiff's view is probably in part true. Scientific opinion has, however, inclined to favour the view of Dr Pavy, that the non-digestion of the living stomach is connected with the circulation through the blood-vessels of the mucous membrane, of alkaline blood, whence there is continually transuding alkaline plasma, which bathes the ultimate anatomical elements of the tissues. The acid gastric juice which could penetrate to these, having its acidity removed, is naturally rendered inert. This view has been supported by the fact that when certain of the arteries of the stomach are tied, the areas supplied by them are liable to perforation, by a process said to be similar to that of post-mortem digestion; more probably, however, perforation depends upon a necrotic process, affecting the anatomical elements of the part concerned.

The Physiological Action of Albumoses and Peptones.

The observations of Schmidt-Mülheim.

Allusion has already been made to the observations of Schmidt-Mülheim and of Fano on the influence of albumoses in checking the coagulation of the blood. The comparative action of albumoses and peptones will now be considered. In a series of researches on the nature and physiological action of the products of the digestion of proteids, Schmidt-Mülheim[1] announced the fact that when peptones are injected into the blood-vessels of living dogs, certain remarkable phenomena are observed, the most important of which are first, that the animal passes into a state of narcosis, resembling chloroform narcosis, accompanied by a great fall of the general blood-pressure; second, that the blood drawn from the blood-vessels has lost its power of spontaneous coagulation. The material employed in these researches was Witte's 'Peptonum siccum,' a commercial preparation, which Kühne's researches have since shewn to be composed of a mixture of albumoses.

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[1] A. Schmidt-Mülheim, 'Zur Kenntniss des Peptons und seiner physiol. Bedeutung' (Aus d. phys. Anstalt zu Leipzig). Du Bois Reymond's *Archiv f. Anat. u. Physiolog.* Phys. Abtheil. 1880, p. 33.

The observations of Fano.

Fano[1], the year after the above interesting results were made known, published observations which confirmed and extended those of Schmidt-Mülheim.

He found, employing essentially the same preparation as Schmidt-Mülheim, that, as a rule, in dogs, the coagulation of blood was prevented by injection of peptones in the proportion of 0·3 grm. for each kilo. of body-weight. Curiously, he discovered that when injected into the blood-vessels of rabbits, no change in the coagulability of the blood occurred. When Fano injected tryptones, *i.e.* antipeptones resulting from the digestion of proteids by trypsin, into the blood of dogs, no change in the coagulability occurred.

Fano found that peptone-plasma could be rendered coagulable by diluting with water and passing CO_2 through it. He also discovered that the lymph of animals whose blood has been rendered uncoagulable by peptones, is also uncoagulable.

The observations of Pollitzer.

When the researches of Kühne and Chittenden had shewn that the commercial peptones, which had furnished the raw material with which Schmidt-Mülheim and Fano had worked, consisted in great part of albumoses, it became obviously necessary to repeat their observations with albumoses and with peptones prepared by the light of recent researches. Accordingly, Dr Pollitzer, working in Kühne's laboratory, undertook the investigation. He found that both albumoses and amphopeptones are possessed of active physiological properties, inasmuch as both classes of bodies induce narcosis in dogs and cats, which is much more enduring in the case of albumoses, probably in consequence of peptones being more readily eliminated, a result connected, doubtless, with their much greater diffusibility. Whilst a sufficiently large dose of any of the albumoses (somewhat more than 0·3 grm. per kilo. of body-weight) is inevitably fatal, peptone never produces a fatal result so long as the kidneys of the animal are intact. Schmidt-Mülheim had found that after the injection of his preparations there was an invariable fall of blood-pressure, and Pollitzer proved that this result follows the introduction of any of the albumoses or peptones except perhaps antipeptone, of which the action is doubtful.

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[1] Fano, 'Das Verhalten des Peptons und Tryptons gegen Blut und Lymphe.' Du Bois Reymond's *Archiv f. Anat. u. Phys.* Phys. Abtheil. 1881, p. 277.

Pollitzer found, like Fano, that antipeptone is without action on the coagulation of the blood. Albumoses possess an action which is very much more marked and constant than amphopeptones.

The period during which the blood remains uncoagulated, after the introduction into the circulation of an albumose, varies between 20 minutes and several days.

Hetero-albumose, in Pollitzer's hands, acted most uniformly, for in no case in which it was used did the blood clot within a period of 24 hours after injection. Seven injections were made with amphopeptones. In three the blood clotted normally; in four its coagulation was delayed for 10, 20, 30 minutes and 12 hours, respectively. Even when blood is mixed with solutions of albumoses after it has been shed, the period of coagulation is usually delayed, sometimes very markedly so[1].

SECT. 15. THE PROCESS OF GASTRIC DIGESTION IN DISEASE.

Before briefly glancing at the principal changes which the normal process of gastric digestion exhibits in pathological conditions of the organism as a whole, and in local affections of the stomach itself, it appears desirable to discuss the interesting question how far gastric digestion is to be considered prominent or essential amongst the phenomena of the alimentary canal and of the organism.

The physician is so constantly brought face to face with cases in which a mere enfeeblement of the functions of the stomach leads to prominent distress and profound malnutrition, and with others in which a local gastric lesion reduces the patient to a state in which life is threatened, and often lost, that the results of experiments performed upon the lower animals, and now to be described and commented upon, appear little short of inexplicable.

Survival of dogs after complete removal of the stomach.

The experiments of Heidenhain have been described, in which the fundus of the stomach, or its pyloric portion, were, as it were, eliminated from the alimentary canal, and, it was pointed out, that after these experiments the animal often survived. The question as to whether the stomach, as a whole, might, in cases of cancer, be removed in its entirety, consistently with the life of the patient, therefore suggested itself. Accordingly Czerny and his pupils, Kaiser and Scriba, carried out the removal of the entire stomach of dogs, and with such remarkable success that, of two dogs operated upon, one survived the operation for some years, regaining perfect health, increasing indeed very greatly in weight, and differing apparently in no respect from a normal animal, when four years after the operation, for the purpose of the investigation, it was killed.

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[1] J. Pollitzer, 'On the Physiological Action of Peptones and Albumoses.' (From the Physiolog. Institute, Heidelberg.) *Journal of Physiology*, Vol. VII. p. 283. A preliminary notice of the results announced in this paper, was first published in the *Verhandl. d. Naturhist. Med. Vereins zu Heidelberg*, N. I. Bd. III. Hft. IV.

The dog referred to was operated upon on Dec. 22, 1876. After the operation, the animal was only fed on small quantities of milk and powdered meat, but after two months it ate the ordinary food of other dogs. Before the operation the dog weighed 5850 grms. On Jan. 22nd its weight had fallen to 4490 grms., but it increased afterwards, so that on Sept. 16th it weighed 7000 grms.

"In Leipzig, in the year 1882, Ludwig and his pupil Ogata were engaged in investigating the functions of the stomach. It occurred to them that it would be interesting to learn what had become of Czerny's dogs. Ludwig wrote to Heidelberg, to Czerny, who answered by sending the dog in a perfectly healthy state to Leipzig. It was in excellent spirits, and ate all kinds of food with a keen appetite. The faeces were normal. In consequence of the abundant food, it had put on weight, and it did not appear to differ in any way from an ordinary dog. With Czerny's consent, the dog was killed in the spring of 1882. The post-mortem shewed that only a very small portion of the cardiac end of the stomach remained, and this was dilated into a small cavity filled with food. The dog had therefore lived for more than five years without a stomach[1]," or, to be more precise, with only a small remnant of its original stomach.

Ogata's experiments[2].

Ogata, working under Ludwig's direction, instead of having recourse to the formidable operative procedure of Czerny, established a duodenal fistula, which permitted the introduction, through the fistula, of an india-rubber ball, connected with a tube, which allowed of the ball being distended with water, so that it shut off the stomach from the duodenum. It was then possible to introduce alimentary substances into the duodenum.

It was found that the introduction of pounded egg and minced flesh into the duodenum, twice daily, sufficed to keep the animal experimented upon, in health, and up to weight. Ogata found that, in the main, digestion proceeded as usual, there being however somewhat less perfect digestion of connective tissue.

These extraordinary results are in agreement with the knowledge which we possess, that the stomach discharges digestive functions which are shared by other organs, and prove that in animals possessed of great vitality, the failure of one organ may lead to such compensatory over-activity of the cooperating organs as suffices, for a time at least, to shield the organism from evil consequences.

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[1] F. F. Kaiser in Czerny's *Beiträge zur operativen Chirurgie*, 1878, p. 141.

This account of the experiments of Czerny and his pupils is quoted from the interestingly written account in Bunge's admirable 'Text-Book on Physiological and Pathological Chemistry.' London, Kegan Paul and Co. 1890. See Lect. IX. p. 167.

[2] Ogata, 'Ueber die Verdauung nach der Ausschaltung des Magens,' *Archiv f. Anat. u. Phys.* Phys. Abtheil. (1883), p. 89.

Whilst these results in no respect justify the conclusion, opposed to all our experience, that the stomach, in man, plays but an unimportant part in digestion, they serve the valuable purpose of impressing upon our minds the great, *perhaps* the paramount, importance of the pancreas in digestion.

The Gastric Juice in Disease.

Methods of collecting the gastric juice or the mixed contents of the stomach.

Our information of the changes which occur in digestion in disease are derived in great part either from the examination of the stomach contents obtained by the act of vomiting, or by collecting the gastric juice, more or less mixed with water or with portions of food, either by employing the stomach-pump or a flexible hollow gastric sound, to empty the stomach some time after food has been taken.

In the accompanying illustrations are shewn (1) the process of washing out the stomach by means of the stomach-pump (Fig. 10), (2) a stomach tube or gastric sound (Fig. 11).

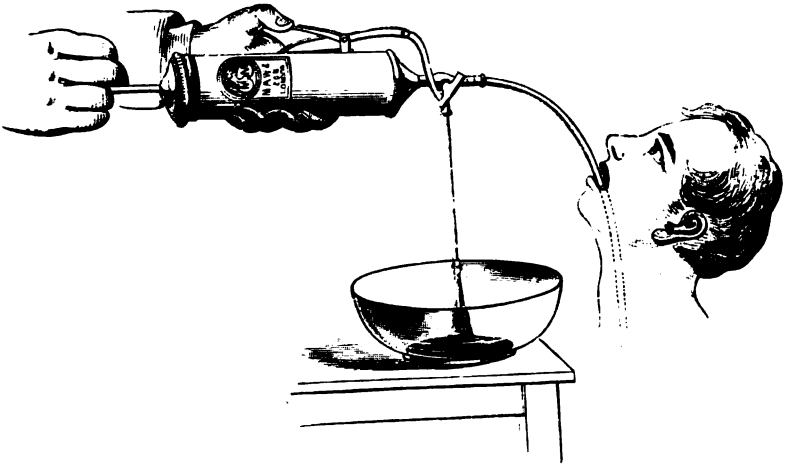

FIG. 10. THE ACT OF EMPTYING THE STOMACH BY MEANS OF THE STOMACH-PUMP. (Maw.)

By appropriate manipulation of the two-way cock, water or other liquid may be aspirated from the basin and thereafter pumped into the stomach, and subsequently aspirated from the stomach and pumped into the basin; the latter operation is represented in the figure.

The gastric sound was first employed by Leube[1] and Külz[2], and has since been greatly employed on the Continent of Europe both for diagnostic and therapeutic purposes.

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[1] Leube, *Archiv f. klin. Medic.* Vol. 33, 1883. 1. Refer to his article in Ziemmsen's *Handbuch d. spec. Pathol. u. Therapie.* Also *Sitzungsberichte d. phys. med. Societät zu Erlangen*, 1871, Hft. 3.

[2] Külz, *Deutsche Zeitschrift f. prakt. Med.*, 1875, No. 27.

The sound consists of a soft india-rubber tube, of about the same thickness as a stomach-pump tube, the inner or stomach end of which is rounded, and, at a short distance from this end, it is perforated by a hole through which the fluid passes into, and out of, the stomach.

This pipe may be attached to a funnel. The tube being inserted into the stomach, *lege artis*, exactly as a stomach-pump tube, some water is poured into the funnel, which is held at this time in an elevated position. On now depressing the funnel the contents of the stomach will be syphoned off and accumulate in the funnel. An ingenious syphon for washing out the stomach is shewn in Fig. 12.

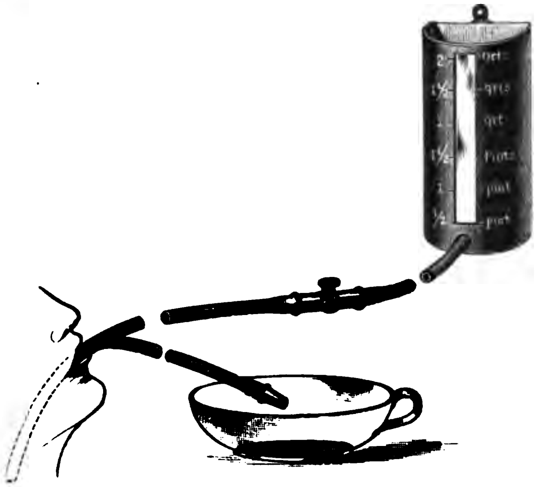

FIG. 12. SYPHON FOR WASHING OUT THE STOMACH. (MAW.)

It is obvious that with the help of such contrivances various kinds of observations may be performed; as, for instance, we may collect the stomach contents mixed with a small quantity of water a known time after a so-called *test meal;* we may collect the stomach contents at any time after the ordinary meals; or we may wash out the stomach with pure water, or water containing various agents.

Test-meals. In experimenting on the digestive powers of the stomach in disease it is very usual with some clinical observers, especially in Germany, to

FIG. 11. A STOMACH TUBE, OR HOLLOW GASTRIC SOUND. (MAW.)

cause patients to take the *test meals*, above referred to, and then to examine the stomach contents, consisting of gastric juice, undigested food and products of digestion, one or several hours afterwards.

The test meal is administered on an empty stomach. Ewald[1] employs for it a dry well-buttered roll with water or weak tea. Leube and Riegel employ a meal of "Wasser-Suppe," "Gries-Suppe" and meat.

On the Influence of Changes in the Acidity of the Gastric Juice in Disease.

Amongst all the changes which the gastric juice exhibits in disease, modifications in the quantity, as well as the nature, of the acid which it contains occupy the first place. All general affections of the organism profoundly depressing its nutrition: the condition of pyrexia, whatever its origin: the zymotic fevers, etc., whilst all tending to diminish or arrest the secretion of gastric juice, are likewise associated with a diminution in the amount of acid which it contains. Several of the functional and organic diseases of the stomach seem to lead to the secretion of a gastric juice abnormally deficient in its normal acid.

The secretion of such a gastric juice is often the starting point for a series of phenomena which profoundly affect the processes of digestion in the stomach, as well as the organism as a whole.

First and foremost, the function of the acid of the gastric juice is, as we have seen, to cooperate with pepsin in the process of proteolysis. Next to this, the acid discharges another function of which the importance to the organism can scarcely be exaggerated, viz. an antiseptic and a disinfecting function; for it would appear that the remarkable antiseptic and deodorizing properties of the gastric juice are intimately associated with, and dependent upon, its acid.

We are continually introducing with our food, into our organism, moulds and yeasts and bacteria, which, but for the action of the acid of the gastric juice, would set up fermentations of various kinds, or which developing within the organism, would be the cause of numerous zymotic diseases from which we are more or less protected. Amongst the numerous protective agencies which are at work preserving the organism against the inroads of putrefactive and pathogenic bacteria, the influence of the gastric juice must therefore not be lost sight of or underrated.

Bunge's views.

Bunge has, in his Text-book, discussed in his habitually interesting, original and instructive manner, the capital importance of the acid of the gastric juice, arguing that the antiseptic action of the juice is even more important than its function as a proteolytic agent. He says:—"A strong point in favour of the view that the antiseptic action of the gastric juice constitutes its chief importance is found in the fact that in a whole series of the lower animals the commencement of the alimentary canal secretes a fluid very rich in mineral acid, but containing no ferment and having no special action on the food[1]." In illustration of his view, Bunge refers to the remarkable observations first made by Troschel and Boedeker[2], which were afterwards fully confirmed by de Luca and Panceri[3] on the acid secretion of Dolium galea, which will be referred to in detail in a subsequent section of the present volume. It appears to the Author that whilst the value of the germicidal and antiseptic action of the gastric juice rests upon well-ascertained facts and cannot be gainsaid, yet the estimate formed by Professor Bunge as to the *preponderating* importance of this function, compared with the proteolytic action of the gastric juice, is an exaggerated one. The acid secretion in Dolium and other invertebrates is related to the external requirements of the creature, and not to its digestive acts; it enables it, in its search for abode and protection to erode the chalky formations which surround it, and we may assume, perhaps, that it also furnishes it with a chemical agent of offence and defence. May we not in the acid secretion of Dolium see an analogy to the venomous secretions which characterise so many vertebrate and invertebrate animals? If indeed we determine to establish our opinion as to the relative importance of the various functions of the alimentary canal on the basis of comparative physiology, we shall be forced to a conclusion very different from that of Professor Bunge. The characteristic digestive process in invertebrata is, as we shall see, one which proceeds in alkaline and not acid media, and which bears most resemblance to the pancreatic digestion of vertebrates.

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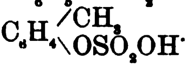
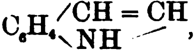
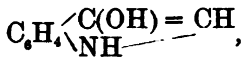
[1] See v. Jacksch, *Clinical Diagnosis*, p. 104.

Antiseptic action of gastric juice influences the aethereal sulphates of urine.

A series of interesting researches has been carried out in which the antiseptic action of the gastric juice is indirectly estimated, under various conditions, by a study of the urinary constituents.

When discussing the putrefactive changes which occur in the intestinal canal, it will be pointed out that, as a result of the action of putrefactive bacteria, there are formed certain phenols, of which the chief are phenol and cresol, and particularly two well-characterised bodies of foul odour, indol and skatol.

The bodies which have been named are in part excreted in the faeces, but in part are absorbed, and entering the blood are excreted as constituents of the urine, in the form of so-called ethereal sulphates. Thus phenol, $C_6H_5.OH$, is principally excreted as phenol-sulphuric acid, $C_6H_5.OSO_2OH$: cresol (methyl-phenol) as cresol-sulphuric acid,  $C_6H_4\begin{matrix}/CH_3\\ \backslash OSO_2OH\end{matrix}$. Indol,  $C_6H_4\begin{matrix}/CH=CH\\ \backslash NH \diagup\end{matrix}$, is first of all converted, in the organism, into indoxyl  $C_6H_4\begin{array}{l}\diagup C(OH) = CH \\ \diagdown NH\end{array}$, and this body enters the urine as indoxyl-sulphuric acid,

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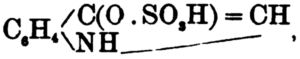
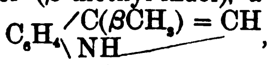
[1] Bunge, *Physiological and Pathological Chemistry*. English translation. Vol. I. p. 158.

[2] Troschel, Poggendorff's *Annalen*, Vol. XCIII. (1854), p. 614, and *Journ. f. prakt. Chemie*, Vol. LXIII. (1854), p. 170.

[3] de Luca et Panceri, *Comptes Rendus*, Vol. LXV. (1887), pp. 577 and 712. An abstract of these researches, written by the Author, appeared in the first volume of the *Journal of Anatomy and Physiology*.



$$C_6H_4\begin{array}{l}\diagup C(O\,.\,SO_3H) = CH \\ \diagdown NH\end{array},$$

Similarly, skatol (β-methyl-indol), a body characterised by a foetid, foetal odour  $C_6H_4\begin{array}{l}\diagup C(\beta CH_3) = CH \\ \diagdown NH\end{array}$, after conversion into skatoxyl, is excreted *chiefly*, as skatoxyl-sulphuric acid.

By repeated investigations it has been shewn that when the contents of the intestinal canal are disinfected, as by the administration of calomel, naphthalin, β-naphthol, &c., the excretion of the ethereal sulphates by the kidneys ceases. Resting on the basis of these facts are the investigations, now to be referred to, in which the disinfecting action of the gastric juice was determined by the estimation of the ethereal sulphates of the urine.

Wasbutzki[1] determined the ratio of the quantity of sulphuric acid present in the form of ethereal sulphates, to the total quantity of sulphuric acid in the urine, in a series of cases where abnormal fermentations occurred in the stomach. In these cases he also determined the nature and amount of the acids in the gastric juice. He found that the ethereal sulphates were increased in amount in cases of acid fermentations of the contents of the stomach due to bacterial action, the hydrochloric acid of the gastric juice being either absent or in greatly diminished amount. In cases, however, of fermentations due to the developement of *torula* in the stomach, he found an absence of ethereal sulphates in the urine. In these cases the gastric juice was abnormally acid, and contained an excess of hydrochloric acid. From Wasbutzki's researches, it would appear, however, that large quantities of lactic and butyric acid in the stomach contents may exert the same influence on the putrefactive bacterial processes in the alimentary canal as is normally exerted by hydrochloric acid.

A. Kast[2] attempted to determine the antiseptic action of the gastric juice by administering such quantities of alkalies to healthy men as sufficed to render their urine neutral or alkaline. He found that under this treatment the ratio of the ethereal sulphuric acid to the total quantity of sulphuric acid excreted was largely increased; and that whenever the acidity of the gastric juice was more or less diminished, the effect was made obvious in this way.

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[1] M. Wasbutzki, 'Ueber den Einfluss von Magengährungen auf die Fäulnissvorgänge im Darmcanale.' *Archiv f. experim. Patholog. u. Pharmakol.*

[2] A. Kast, 'Ueber die quantitative Bemessung der antiseptischen Leistung des Magensaftes.' *Festschrift z. Eröffnung d. Allgem. Krankenhauses zu Hamburg-Eppendorf*, 1889 (Sep. Abd.). Abstracted by Professor Andreasch, in Maly's *Jahresbericht* for 1889. Vol. xix. p. 271.

It is through failure to arrest the developement of the organisms which are the cause of acetic, lactic, butyric and similar fermentations that the feebly acid gastric juice secreted in morbid conditions of the organism and of the stomach reacts injuriously upon its functions, bringing about catarrhal conditions which are associated with various dyspeptic manifestations. Amongst these manifestations are prominently observed such as are due to the action of the organic acids which are the products of abnormal fermentations.

Action of acid of gastric juice in checking fermentation.

Ewald pointed out that the hydrochloric acid of normal gastric juice possessed the property of checking the production of lactic and acetic acids[1]. His statements have received confirmation from the researches of Hirschfeld[2] and of Felix O. Cohn.

The former writer added artificial gastric juice, containing varying quantities of hydrochloric acid, to culture solutions which had been inoculated with *Bacill. acid. lact.* (Hueppe) or with sour milk, and determined by titration the quantity of lactic acid formed. He found that from 0·01 to 0·02 per cent. of HCl sufficed to slow, very powerfully, the developement of lactic acid; the same effect was observed in the case of the acetic fermentation.

Felix O. Cohn[3], from a series of experiments similar to those of Hirschfeld, arrived at the following conclusions: 1st. That pepsin does not inhibit the formation of acetic and lactic acids. 2nd. That even mere traces of hydrochloric acid hinder the acetic fermentation. 3rd. That whilst hydrochloric acid hinders the developement of the lactic fermentation, as much as 0·7 per cent. is required in order to arrest it.

A considerable number of bacteriological investigations have been made on the micro-organisms which occur in the healthy human stomach, but the results have been by no means concordant. Capitan and Moreau[4] found only three micro-organisms in their examinations of the stomach contents of thirty healthy human beings; two of these organisms were varieties of yeast and one a bacillus, somewhat broader than the tubercle bacillus, forming colonies and liquefying gelatine. Abelous[5], on the other hand, succeeded in discovering 16 micro-organisms, of which 7 are known, to wit, *Sarcina ventriculi*[6], *Bacillus pyocyaneus*, *Bacterium lactis aerogenes*

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[1] Ewald, *Berliner Klin. Wochenschrift* 1886, No. 48.

[2] E. Hirschfeld, 'Ueber die Einwirkung des künstlichen Magensaftes auf Essigsäure- und Milchsäuregährung.' *Pflüger's Archiv*, Vol. XLVII. 510—542.

[3] Felix O. Cohn, 'Ueber die Einwirkung des künstlichen Magensaftes auf Essigsäure- und Milchsäuregährung.' *Zeitschrift f. physiolog. Chemie*, Vol. XIV. 74—105.

[4] Capitan et Moreau. *Comptes Rendus de la Société de Biologie*, 41. 25.

[5] Abelous. *Comptes Rendus de la Société de Biologie*, 41. 86.

[6] Sarcina ventriculi received the name which it now bears from John Goodsir, who first discovered it in the vomited matters of certain cases of dyspepsia (*Edinburgh Medical and Surgical Journal*, Vol. LVII. (1842) p. 430). Sarcina is an alga, and after its discovery by Goodsir was recognised as identical with the alga discovered by Von Meyen in 1829 and named by him *Merismopedia punctata* (see Article 'Verdauung' in Wagner's *Handwörterbuch*).

(von Eschricht), *Bacillus subtilis*, *B. mycoides*, *B. amylobacter*, *Vibrio rugula*; in addition to these well-known organisms, there occurred also one coccus and eight bacilli. All these organisms, according to Abelous, resist the action of artificial gastric juice, containing 1·7 per thousand of HCl[1].

Action of the gastric juice on pathogenic organisms.

The action of the gastric juice on pathogenic organisms naturally offers the greatest interest, and has been the subject of many investigations.

Amongst the first to deal with the subject in a thoroughly satisfactory manner were Falk and Frank. Falk[2] found that the Bacillus anthracis (the pathogenic organism which occasions the so-called '*splenic fever*' of cattle, '*Milzbrand*' or '*Charbon*') is not acted upon by the saliva, the pancreatic juice or the bile, but that it is readily destroyed by the action of the gastric juice, although the spores which the bacillus may contain usually escape destruction. Falk found that the Tubercle bacillus was not affected by any of the digestive juices, including the gastric juice, though it readily succumbs to the putrefactive process.

Frank's researches[3] fully corroborated the observations of Falk, both in the case of the Tubercle bacillus and in that of the B. anthracis, and, like Falk, Frank came to the conclusion that the action of the digestive juices opposed no very general and effectual resistance to the inroads of infecting pathogenic organisms.

Since the date of these researches many observers have experimented in the same direction, and with the more interesting of their results we shall now deal.

The experiments and observations of Nicati and Rietsch and of Koch himself have established that the Comma bacillus of Koch, which has now been proved, beyond the possibility of doubt, to be the pathogenic organism of Asiatic cholera, is readily destroyed by the action of the gastric juice, and indeed by dilute solutions of hydrochloric acid. We are thus enabled to explain satisfactorily the difficulty which has been experienced in communicating cholera to animals (and even to man) by the administration of pure cultures of the Comma bacillus. The experiments of Nicati and Rietsch[4] have however shewn that if the stomach be washed out with alkaline solutions, so as to abolish the acid reaction imparted to it by the gastric juice, the introduction of cultures of the Comma bacillus is in some cases followed by the invasion of symptoms akin to those of cholera; the same result follows the introduction of pure cultures into the intestine. These interesting facts appear, at first sight, to stand in opposition to the belief or, rather we should say, to the decisive experience of the most careful and trustworthy observers, that the *contagium* of cholera is in general introduced into the organism through the alimentary canal. A little reflection will, however, readily dispose of the apparent inconsistency between the results of the experimental researches of R. Koch[1] and of Nicati and Rietsch and the experience of physicians.

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[1] The reader may wish to refer to a paper by W. de Bary entitled 'Beiträge zur Kenntniss der niederen Organismen im Mageninhalte.' *Archiv f. exp. Pathol. und Pharmakologie*, Vol. xx (1885), p. 243.

[2] Falk 'Ueber das Verhalten von Infectionsstoffen im Verdauungscanale,' Virchow's *Archiv*, Vol. xciii (1883), p. 117.

[3] Edmund Frank 'Ueber das Verhalten von Infectionsstoffen gegenüber den Verdauungssäften,' *Deutsche Med. Wochenschrift*, 1884, No. 20.

[4] W. Nicati and N. Rietsch, *Revue Scient.* 1884, p. 658; *Comptes Rendus*, Vol. xcix. p. 921.

Drinking water contaminated by the dejections of cholera patients appears to be, in the immense majority of cases, the medium of infection. Water is continually introduced into the stomach at a time when digestion is in abeyance and the organ free from gastric juice; moreover, the dilution which any trace of acid must undergo when considerable quantities of water are drunk will eventually serve to protect the cholera bacilli of which the water is the vehicle.

The bacilli which are apparently directly, or through the products of their vital activity, the *materies morbi* of typhoid (B. typhi), of diphtheria (Löffler's B.), and of tetanus (B. tetani of Kitasato) are all, apparently, injuriously affected or destroyed by digestion in gastric juice. In the presence of albuminous substances, the hydrochloric acid of the gastric juice, which combines with them, loses to some extent its efficacy as a germ destroyer, and the organisms, above referred to, may thus retain their pathogenic activity.

GASTRIC DIGESTION IN SPECIAL DISEASES.

1. *Gastric Digestion in Fevers.*

This has been made the subject of experimental inquiries and direct observations. We may say, in general, that in febrile affections the quantity of gastric juice is diminished: that the hydrochloric acid is often altogether absent or very much diminished: that the pepsin on the other hand is not altered[2]. The reaction of the gastric juice was sometimes found neutral or even alkaline (Uffelmann), and in a case reported by von den Velden[3] the acid reaction was due to lactic acid (the case was one of typhoid).

With these results agree well the facts ascertained by Manassein[4], who made animals febrile by injecting putrid matter into the blood and found that the collected gastric juice only possessed digestive properties when HCl was added to it.

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[1] R. Koch, *Deutsche med. Wochenschrift*, 1884, No. 45.
[2] Leube, *Volkmann's klin. Vorträge*, No. 62.
[3] *Berlin. klin. Wochenschrift*, 1877, No. 42.
[4] Virchow's *Archiv*, Vol. 56, p. 413.

More recent observations however shew that there are exceptions. Sassezkis[1] examined the gastric juice in nine persons suffering from febrile affections and found the HCl only very much diminished in those cases where there was, besides, a very marked dyspepsia. Edinger[2] examined several cases of fever (one case of typhoid with vomiting, two cases of recurrent fever, and one of intermittent) and found hydrochloric acid present in all.

2. *Gastric Digestion in Dyspepsia.*

Clinically considered, dyspepsia, or indigestion, denotes a complex of symptoms common to various disorders, characterized principally by abnormalities in the gastric digestion. Having regard to these symptoms we may still adhere to the time-honoured classification of dyspepsia into flatulent dyspepsia, acid dyspepsia and atonic dyspepsia.

Flatulent Dyspepsia. The causes of this form of indigestion are numerous, and may be due either to an improper diet, either as regards quality or quantity: to acute or chronic catarrh of the stomach, to atrophy of the glandular elements, changes in the muscular coat, presence of ulcer, cicatrix of an ulcer, or cancer of the stomach, to a diseased condition of other parts of the digestive tract (insufficient mastication, disturbances of salivary, pancreatic or biliary secretion): to general diseases (anaemia, gout, tuberculosis), or to diseases of other organs (diseases of uterus, cardiac diseases, nervous disorders).

The changes in gastric digestion are due to fermentative changes which the ingested food undergoes and are brought about either by the character of the food, the insufficiency of hydrochloric acid in the gastric juice, or the prolonged stay of the food in the stomach; hence this form of dyspepsia, when very marked, is almost always associated with dilatation of the stomach.

The only points, which we need consider, concern: (1) vomited matter, (2) condition of the gastric juice, (3) flatulence.

1. Vomited matter. Vomiting is a usual symptom of this form of dyspepsia; if but little dilatation of the stomach exists, the vomited matters are small in quantity, consist of food either unaltered or often in a state of fermentation, frothy and smelling of yeast, or of an acid or rancid smell; sometimes there is an admixture of bile, at other times of masses of mucus.

Microscopically we find large masses of sarcina and of torula, besides the elements of undigested food.

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[1] *St Petersburger med. Wochenschrift*, 1879.

[2] *Deutsch. Archiv f. klin. Med.* Vol. XXIX. p. 565.

Chemical examination shews the presence of acetic, lactic, and butyric acids. The hydrochloric acid is either altogether absent or present only in small quantities; pepsin, according to Kussmaul, is always present. Kussmaul found it absent only in one case, in which enormous masses of a fungus were contained in the stomach.

The acids present are the result of fermentation of the ingested carbohydrates, as was first pointed out by Frerichs; this would explain, on the one hand, the presence of lactic and butyric acids (as well as CO_2 and H), while on the other hand the alcoholic and acetous fermentations would result in the appearance of alcohol, CO_2 and acetic acid as final products. Both these forms of fermentation may go on at the same time[1]. Besides these acids, we find unaltered albumen, sugar, and starch present in the vomited matter.

If dilatation of the stomach be excessive, or if almost complete mechanical obstruction exist, as in cases of cancer of, or cicatrix at the pylorus, the vomited matters, or the stomach contents which can be withdrawn by the stomach tube, may amount to a large volume (2—4 litres). When allowed to stand, they usually separate into three layers: an upper one, consisting of frothy mucus, a subjacent layer of liquid, and a sedimentary stratum at the bottom, containing solid masses and particles.

The gases which are evolved in this form of dyspepsia have sometimes a composition similar to that of the atmospheric air, except that they contain more CO_2; when, however, the fermentation process is fully developed they consist chiefly of H and CO_2.

According to Popoff[2], in some cases, an inflammable gas is evolved (Frerichs, Friedresch, Ewald[3]), which proved to be marsh gas. Hoppe Seyler believes that this gas is derived from the intestines and is not formed in the stomach (?)

The following numbers exhibit the composition of the mixed gas, in cases of this kind.

	Frerichs	Popoff	Schultze	
CO_2 in 100 parts	20·57	12·82	26·56	in 100 parts.
H	20·57	32·32	32·30	
CH_4	10·75		0·34	
O	6·52	7·90	7·36	
N	41·39	46·96	33·44	

In the cases we are now considering, pyrosis is often a symptom in addition to vomiting.

If the *gastric juice* be examined (by means of stomach-pump or stomach tube) several hours after the patient has vomited, it is found to contain only traces of hydrochloric acid, while pepsin is present.

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[1] Schultzen, *Archiv f. Anat. u. Physiolog.* 1864, p. 591.

[2] *Berl. klinische Wochenschr.* 1870, Nos. 38 and 40.

[3] *Archiv f. Anat. u. Physiol.* 1874.

Acid Dyspepsia. This form of dyspepsia may often occur in perfectly healthy persons; at other times it is associated with catarrh of the stomach and other disorders. The vomited matter often consists of a clear fluid, mixed with more or less mucus and is of intensely acid character. It has been supposed by most observers that the acidity is due to lactic acid (Richet, *loc. cit.*); this seems to be especially the case if much mucus be present. McNaught[1] found however in several cases the acidity due to increased HCl (often amounting to 2·7 per mill.). Sir W. Roberts has fully confirmed the observation of McNaught. He has drawn particular attention to the sudden paroxysmal attacks of cramp of the stomach, which are apt to occur in the course of acid dyspepsia and for which he would retain the term of '*pyrosis*,' which has been somewhat vaguely employed. In connection with these paroxysmal attacks of cramp, a sudden gush of saliva into the mouth is very frequent, and Sir W. Roberts has found that the saliva is in some cases of remarkable alkalinity, able to neutralise 0·125 per cent. of HCl. The secretion of this alkaline saliva is the concomitant of the pouring out of a highly acid gastric juice[2].

Atonic Dyspepsia. As this form of dyspepsia is often dependent on general malnutrition, due to some altered innervation of the secreting apparatus (nervous dyspepsia), the changes in the gastric digestion are similar to those seen in anaemia. In these cases the gastric secretion is deficient in quantity, the hydrochloric acid being often only found in traces. In some cases, the reaction of gastric juice is however distinctly acid, but this is due to the presence of lactic acid in large proportions. As in atonic dyspepsia, Pavy often found increased secretion of saliva, this may possibly account for the abnormal fermentation and with it the presence of lactic acid. In many cases however the salivary secretion is not increased (Wilson Fox).

Dyspepsia in Phthisis pulmonalis. Any cause which profoundly disturbs the nutrition of the body may act as a predisposing cause of consumption. Disturbances of digestion are very frequently precursors of the disease, and presumably favour the infection of the organism. Such being the case, we should expect to find abnormalities in gastric digestion in a considerable proportion of cases of pulmonary consumption.

If we consider, moreover, that as a result of the tubercular processes a condition of secondary anaemia is very frequent in phthisis, we shall be led to surmise that gastric digestion will be found to be abnormal even in a larger number of cases than those in which dyspepsia appears to have been a marked etiological factor. The observations made by Brieger[3], bear out the surmise.

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[1] *Brit. Med. Journal*, Dec. 30, 1882.

[2] Roberts, *Lectures on Dietetics and Dyspepsia*, London, 1885. See p. 81 *et seq.*

[3] Brieger 'Ueber die Functionen des Magens bei Phthisis Pulmonum,' *Deutsche med. Wochenschrift*, 1889, No. 14.

Brieger made 300 examinations of the gastric juice, secreted after test meals, in a series of 64 cases of Phthisis. In severe cases, only about 16 per cent. exhibited a normal digestion, the remainder suffering from a more or less marked insufficiency of the digestive process. In 9·6 per cent. of these cases there was absence of the normal constituents of the gastric juice.

In cases of medium gravity, 33 per cent. alone secreted a normal gastric juice, all the remainder suffering more or less seriously from gastric affections, while in 6·6 per cent. the secretion of a juice occurred in which the normal constituents were absent.

The observations of Brieger have been fully confirmed by Hildebrand[1], and agree in the main with the experience of physicians specially conversant with phthisis.

3. *Gastric Digestion in other Diseases of the Stomach.*

Acute Gastric Catarrh. The vomited matter consists of undigested food often mixed with bile and mucus.

The gastric juice is diminished in quantity, and if there be much fever the hydrochloric acid is absent.

The stomach is also lined by large quantities of a thick tenacious mucus. (Direct observation by Beaumont in the case of A. St Martin.) In cases of alcoholic origin the stomach contents may contain large quantities of acetic acid.

In acute gastritis, produced experimentally by alcohol, etc., Ebstein and Grützner[2] found, on examining the mucous membrane of the stomach hardened in alcohol, that the chief cells were small, granular, and stained deeply with carmine; presented, indeed, in a marked degree the appearance of 'active' gland cells, a granular and shrivelled condition of the chief cells; he believed this to be due to a continuous formation and secretion of very small quantities of pepsin. The administration of large quantities of alcohol produce all the symptoms of acute gastric catarrh with diminished secretion of gastric juice.

Chronic Gastric Catarrh. We have here either the symptoms of flatulent dyspepsia with the changes described above, or, in cases of very long standing, those of atonic dyspepsia.

Gastric Ulcer. The changes in gastric digestion vary according to the symptoms.

At times, gastric digestion is perfectly normal, at other times we have symptoms of atonic dyspepsia with corresponding change in the secretion; when ulcer has undergone cicatrization, and is so situated as thereby to cause mechanical obstruction to the passage of food, we have the symptoms of flatulent dyspepsia. "According to Riegel, *in cases of round ulcer of the stomach, the acid constituents of the gastric juice are greatly in excess*, and the fact has been further established by the observations of Korczynski, Jaworski and many others[1]." The vomiting of blood is the most prominent symptom; the blood vomited is either red, fluid, unaltered (erosion of a large blood-vessel) or coagulated and dark, fluid and acid (change brought about by contact with the gastric juice).

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[1] Hildebrand, 'Zur Kenntniss der Magenverdauung bei Pthisikern,' *Deutsche med. Wochenschrift*, 1889, No. 15.

[2] Pflüger's *Archiv*, Vol. VI. p. 1 and Vol. VIII. p. 122, see also Grützner, *Neue Untersuchungen über die Bildung und Ausscheidung des Pepsin.*

Cancer of the stomach.

The changes in gastric digestion will vary here also, according to the symptoms, and any of the changes described under the different forms of dyspepsia may be observed. The vomited blood appears here, from its prolonged contact with the gastric juice and from the fact that the haemorrhage is rarely profuse, in the form of the coffee-ground vomiting.

Von den Velden[2] found in 8 cases of cancer of pylorus (in 5 of which the diagnosis was verified post mortem) that the gastric juice obtained by means of the stomach-pump, contained no trace of hydrochloric acid; in all of these there was considerable dilatation of stomach, but no pyrexia, and he believes the absence of the HCl to be an important point in the differential diagnosis between cancer and other affections of the stomach. In all these cases he examined the gastric juice repeatedly, but always with the same result. Rosenstein[3] could not verify these observations. In one case at the Manchester Infirmary where the symptoms during life were those of pyloric cancer and where the HCl was always found absent in repeated examinations, the post mortem examination shewed the presence of a sarcomatous tumor at the upper end of jejunum, while the stomach was free from disease.

In general the experience of physicians appears to be that in cancer of the stomach the colour reactions due to hydrochloric acid are either feeble or absent, and the non-discovery of hydrochloric acid is one point to be taken into consideration in establishing a diagnosis. In advanced cases of cancer of the stomach, the quantity of pepsin formed appears to be exceedingly scanty, and the rennet-ferment, which is a constant ingredient of normal gastric juice, is often entirely wanting.

Amyloid degeneration of stomach.

Edinger and Riegel (*loc. cit.*) found in 2 cases total absence of hydrochloric acid.

Chronic atrophy of stomach.

It was stated by Fenwick that in this affection pepsin is absent or present only in very small quantities, and the observations of W. Jaworski confirm the assertion[4].

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[1] v. Jacksch, *Medical Diagnosis*, p. 96.

[2] *Deutsch. Archiv f. klinische Medicin*, Vol. XXIII. p. 369.

[3] See Article 'Dyspepsia' in Eulenburg's *Cyclopaedia*.

[4] W. Jaworski, 'Zur Diagnose des atrophischen Magencatarrhs,' *Wiener med. Presse*, 1888, Nos. 48 and 49.

SECT. 16. DIRECTIONS FOR LABORATORY WORK CONNECTED WITH GASTRIC DIGESTION.

1. *Determination of the Specific Gravity of Gastric Juice.*

Follow method described in Vol. I., p. 174.

2. *Determination of Total Solids and fixed Mineral Matter.*

Follow method described in Vol. I., p. 177.

3. *Preparation of an Artificial Gastric Juice.*

Follow methods 1 and 4 described at pp. 82 and 83.

4. *Determination of the degree of Acidity of the Gastric Juice, or of the filtered Contents of the Stomach.*

It is usual to express the acidity of the gastric juice as equivalent to n grm. of HCl in 100 or 1000 parts.

In order to make the determination there are required:

(1) A burette say of 30 or 50 c.c., divided into tenths of a cubic centimetre.

(2) A pipette which delivers 10 cubic centimetres; it is convenient to have a second which delivers 5 c.c.

(3) Beakers.

(4) A decinormal solution of sodium hydrate.

(5) A neutral solution of litmus (see Vol. I., p. 176).

Process. The burette is filled to the top of the graduation with the decinormal soda solution. 10 c.c. of the gastric juice of which the acidity is to be determined are placed in a beaker, and 1 c.c. of the perfectly neutral solution of litmus added.

The solution of caustic soda is then allowed to flow in until the red litmus is changed to blue. The volume of the soda solution used is then determined. If n be the number of cubic centimetres of decinormal soda used, and x the amount of acid expressed as HCl in 1 litre of the gastric juice,

$$x = n \times 0{\cdot}00365 \times 100.$$

5. *Determination of the presence of free Hydrochloric Acid, by Colour Tests.*

Test separate small quantities of the gastric juice or of the fluid filtered from the contents of the stomach with the various reagents described in the text (see page 92, *et seq.*), but especially with alcoholic solution of OO Tropaeolin (see p. 94).

[The following is a reprint of the directions for experiments on the detection of small quantities of acids, and on the action of acid, alkali and peptone in modifying the action of ptyalin, drawn up by Mr J. N. Langley, F.R.S., for the use of students attending his advanced Practical Course in the University of Cambridge:

"2. *Detection of small amounts of free HCl.* Tropaeolin OO is changed in colour much more rapidly by free acids than by acid combinations, so that whilst small quantities of either give an acid reaction with litmus, an acid reaction with tropaeolin OO is given by the former only.

"(*a*) The most delicate method of detecting small quantities of free acid is the following: a saturated solution of tropaeolin OO is made in strong spirit, a drop of this is evaporated on a porcelain plate which is placed in a chamber at about 40° C., and to the dried tropaeolin is added a small drop of the fluid supposed to contain free acid; at once, or as the fluid evaporates, the yellow tropaeolin becomes of a violet or purple tint if free acid is present. HCl 0·005 p.c. can thus be detected.

"(*b*) When the percentage of HCl is somewhat greater than this, it is simpler to pour a little of the solution into a test tube, containing a small amount of a saturated aqueous solution of tropaeolin OO, when the yellow colour is changed to orange. About 0·01 p.c. HCl and 0·06 p.c. lactic acid can thus be detected: the colour produced by lactic acid is removed by ether, that produced by HCl is not, unless the amount of HCl be very small.

"(*c*) Paper soaked in tropaeolin OO and dried becomes brownish and, on drying, lilac or violet when a drop containing free acid is placed on it.

"3. Methyl-violet is also used by method (*b*) to detect small quantities of free acid, this colour is changed to blue in the presence of not less than 0·05 p.c. of HCl or 0·5 p.c. of lactic acid.

"4. To a few c.c. of 0·01 p.c. of HCl, a drop of which gives a bright violet mark with tropaeolin OO (method *a*), add a little peptone, the mixture gives no acid reaction with tropaeolin; a similar result is produced by adding serum, or white of egg, probably in consequence of the globulin in these fluids; myosin and certain other proteids act in the same manner.

"5. Take 10 c.c. of 1 p.c. solution of peptone, it will probably be acid to litmus; if so, neutralise it with Na_2CO_3, noting the volume added. Add HCl, 0·1 p.c., from a burette, until a drop of the mixture gives a violet mark with tropaeolin OO; the mixture then contains 0·005 p.c. of *free* HCl. The amount of HCl added, minus the amount of free HCl, gives the amount of the acid which has combined with the peptone; from this, the percentage of HCl taken up by the peptone should be calculated thus: suppose 5 c.c. of HCl (·1 p.c.) is the amount taken up by the peptone, then the percentage is $\frac{5 \times \cdot 1}{10 \times 1} \times \frac{100}{1} = 5$.

"6. *Effect of acid, alkali, and peptone slightly acid, on ptyalin.*

"The ptyalin solution may be prepared thus:

"(*a*) Chop up a ptyalin containing salivary gland, e.g. the parotid of a rabbit, add about 100 c.c. of water, leave at 39° C. for an hour or two. Filter, neutralise, and, if necessary, filter again. (*b*) Neutralise freshly collected saliva, dilute 10 times and filter.

"Take

	Ptyalin Solution.		Water.		Starch 3 p.c.	
(1)	10 c.c.	+	8 c.c.	+	2 c.c.	
(2)	"	+	3 c.c.	+	"	+ 5 c.c. of HCl (0·2 p.c.).
(3)	"	+	0 c.c.	+	"	+ 2·5 c.c. HCl (0·4 p.c.) + 5·5 c.c. sol. of peptone (10 p.c.)
(4)	"	+	3 c.c.	+	"	+ 5 c.c. Na_2CO_3 sol. (0·2 p.c.).
	a		*b*			

"(*a*) and (*b*) should be measured out in different sets of test tubes and the (*b*)s added to the respective (*a*)s as nearly as possible at the same time. (This should be done in all similar experiments.)

"Place the test tubes in a water bath at about 39° C., and from time to time take a drop from each, and add a drop of rather dilute iodine solution on a porcelain plate. As the starch passes through soluble starch, and dextrin to achro-dextrin and sugar, the colour of the mixed drops becomes a transparent blue, then passes into violet, red-brown, light yellow, and finally iodine gives no colour reaction with the solution. At any stage a definite portion may be taken from each solution and tested for sugar. It will be found that the conversion of starch to sugar is rapid in (1) and (3), slower in (4) and hardly takes place at all in (2). In a similar way it may be shewn that peptone saturated with acid almost or entirely prevents the action of ptyalin on starch."]

6. *Determination of the amount of free Hydrochloric present* (Richet's[1] Modification of Schmidt's Method).

Divide the gastric juice in four portions, which are to be accurately weighed.

a. In the first, determine the acidity expressed as HCl by titration with decinormal solution of soda.

b. To the second, add pure nitric acid and then solution of silver nitrate. Collect the silver chloride, wash, dry, ignite, and weigh, and thus determine the total chlorine.

c. To the third, add a little sulphuric acid, evaporate to dryness, ignite and weigh. Assume that the substance weighed, which consists of the sulphates of all the bases present, is composed entirely of sodium sulphate; calculate on this assumption the amount of sodium present, and therefore the amount of chlorine which would be required to combine with the bases present on this assumption.

d. The fourth is mixed with a boiled out solution of potassium or sodium hydrate, and placed under a bell-jar, together with a capsule containing a measured volume of very dilute standard sulphuric acid. After three or four days, the acidity of the standard acid is determined, and from the diminution which has occurred, the amount of ammonia which has been evolved by the gastric juice is ascertained.

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[1] Richet, *Du Suc Gastrique*, p. 33.

In calculating the results, all the ammonia found is calculated as existing in combination with chlorine. The amount of chlorine which was required to combine with ammonia and with the other bases (See *c.*), reckoned as sodium, is then found, and is deducted from the total amount of chlorine found by operation *b.* The difference represents the chlorine existing as free HCl, of which the amount is then calculated.

The process above described is one which should not be undertaken by any one who is not thoroughly familiar with the methods of inorganic analysis. This remark applies still more forcibly to the original process of Carl Schmidt, for which the reader is referred to the original memoir[1].

7. *Determination of the proportion of acids soluble in water and ether.*

It has been stated (p. 96) that valuable information as to the nature of the acid of the gastric juice has been obtained by Richet by employing Berthelot's method of determining the so-called *coefficient de partage.*

For carrying out this method there are required,

(1) An accurately graduated burette.
(2) A pipette which delivers say 25 c.c.
(3) Two pipettes which deliver 10 c.c. To the upper end of one at least of these pipettes is attached an india-rubber tube with a pinch-cock.
(4) One or two bottles with accurately ground stoppers.
(5) Pure ether.
(6) Absolute alcohol.
(7) Standard normal solution of soda.
(8) Litmus solution.
(9) Beakers.

Process. As much of the gastric juice as can be spared, say 25 or 50 c.c., is placed in the glass bottle, and there is then added to it an equal volume of pure ether. This must be free from acid reaction, and it must have been shaken with distilled water so as to saturate it with water.

The temperature of the liquid in the bottle is taken, and then the contents are subjected to a series of vigorous agitations, which may be counted; 500 suffice. The temperature is again taken, and for practical purposes the mean of the two readings (i.e. of that before and that after the agitation) may be taken as indicating the temperature during the experiment. The bottle is then set aside for a few minutes, and a measured volume, say 10 c.c., of the lower watery stratum, and an equal volume of the upper ethereal solution are carefully withdrawn by the aid of the two pipettes previously referred to as fitted with india-rubber tubes and pinch-cocks. The acidity of the liquids is determined with the aid of a decinormal solution of baryta or soda; the former is preferred by Berthelot and Richet, but for reasons which do not appear sufficient.

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[1] Bidder und Schmidt, *Die Verdauungssäfte und der Stoffwechsel.* Mitau und Leipzig, 1859, p. 44.

The 'coefficient de partage' is found by dividing the volume of standard alkaline solution required to neutralize a given volume, say 10 c.c. of the aqueous, by the volume required to neutralize an equal volume of the ethereal solution.

Before determining the acidity of the ether, the liquid is diluted with say 10 c.c. of absolute alcohol; there is thus obtained an alcoholic-ethereal liquid which is miscible with water.

After the first determination, the process of shaking the gastric juice and ether may be repeated a second or even a third time, and also the titrations.

In cases where the exact 'coefficient de partage' of the acids soluble in ether is desired, the ethereal liquid obtained by agitation should be shaken with water and the coefficient be again determined.

8. *Determination of the Peptic activity of different samples of Pepsin or of Solutions containing Pepsin.*

As we cannot separate pure pepsin so as to ascertain its amount, we are obliged to judge of the relative richness in pepsin of different preparations by determining their relative activity. We may do so either by observing the relative amounts of a proteid which can be dissolved in a given time, or by determining the relative times occupied in the solution of a given amount of proteid.

Method of Bidder and Schmidt and others.

In this as in all other cases, a digestive liquid must be first made by mixing a known weight or volume of each of the preparations under examination with water containing either one or two parts of HCl per 1000.

The same volume of each digestive liquid is taken and placed in an incubator, and to each there is then added the same weight of hard-boiled white of egg cut in pieces of approximately the same size and shape. A portion of the same sample of boiled white of egg is analysed so as to determine the proportion of solid matter which it contains. After, say 24 hours, the liquids are filtered and the undigested white of egg in each case is dried and weighed. In this way is found the amount of albumin which has been dissolved in each case, and this will represent the relative peptic activities of the preparation employed.

Brücke's method.

A known weight or volume of the preparations to be compared is mixed with water and hydrochloric acid, so as to yield solutions which contain exactly 1 part of HCl in 1000. At the same time an aqueous solution of HCl of the same strength is prepared. Seven mixtures of each of the digestive liquids with various proportions of the acidulated water are then made and placed in seven separate vessels, the proportion of digestive liquid to water being arranged as follows:—

(1) 16 : 0, (2) 8 : 8, (3) 4 : 12, (4) 2 : 14, (5) 1 : 15,
(6) 0·5 : 15·5, (7) 0·25 : 15·7.

Thus if we had *n* samples of pepsin to examine, we should have *n* sets of seven vessels, each set containing the dilutions according to the above plan.

Now, into each of the vessels is placed a flocculus of well-washed blood-fibrin, and all are placed in an incubator at about 40° C.

The vessels are then closely observed in order to find those of the different sets in which digestion has proceeded to the same extent. If we had, for instance, two sets *A* and *B*, and we found that in vessel 1 of set *A* digestion occurred in the same time as in vessel 2 of set *B*, we should conclude that the digestive activity, which is, within wide limits, proportional to the quantity of pepsin, of *B* was twice as great as that of *A*.

For all the precautions to be followed in employing this method the reader is referred to the description given by its author[1]; it has now been generally superseded by the methods to be described below.

Grünhagen's method[2]. Well washed blood-fibrin is placed for several hours in water containing two parts of HCl per litre. When the fibrin has swollen, it is placed upon filters to drain. If several solutions of pepsin have to be examined, a given weight of the swollen fibrin is placed upon as many filters as there are solutions, and then a measured volume, say 1 c.c., of each of the liquids is poured over the contents of a corresponding funnel. After some minutes, the contents of the funnel begin to dissolve, as is evidenced by liquid beginning to drop from the funnel. The relative peptic activity may be judged of by counting the drops which fall in a given time, or by the volume of liquid which is collected in a given time, or by the time occupied in the complete liquefaction of the whole mass.

Thus, to take an example. In one of v. Wittich's experiments[3], in which he was comparing the relative richness in pepsin of a glycerin extract of the mucous membrane of the fundus and of the pylorus, designating the first 1 and the second 2: he added 1 c.c. of the extract in each case to swollen fibrin placed in two funnels, which were maintained at the temperature of the room. In 1 (fundus) dropping began in two minutes, and at the end of two hours 13 c.c. of fluid had been collected. In 2 (pylorus) dropping began in ten minutes, and at the end of two hours 4·5 c.c. of fluid had been collected.

Grützner's method. Although no very accurate way of testing the relative amounts of pepsin contained in two extracts exists, the one which is most generally useful is Grützner's Colorimetric

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[1] Brücke, 'Vorlesungen,' "Quantitative Bestimmung des Pepsins," p. 302 et seq.

[2] Grünhagen, "Neue Methode die Wirkung des Magen-Pepsin zu veranschaulichen und zu messen." Pflüger's *Archiv*, Vol. v. (1872), p. 203.

[3] v. Wittich, "Das Pepsin und seine Wirkung auf Blutfibrin." Pflüger's *Archiv*, Vol. v. p. 435.

Method. The directions for carrying out, which follow, are quoted verbatim from those drawn up by Mr Langley for the use of his Advanced Practical Class.

"Wash freshly collected fibrin in a stream of water flowing from a tap for 5 or 10 minutes and chop into small pieces; place them in a large quantity of water until the next day, when any pieces which are still coloured with haemoglobin, or which have clumps of fatty matter adhering to them, should be thrown away.

"Place the fibrin in carmine prepared thus:—to 1 grm. of carmine add 1 c.c. of ammonia, mix well and add 400 c.c. of water, and stir. If the mixture smells strongly of ammonia it should be placed aside until it ceases to do so.

"The fibrin should be steeped in the carmine solution for a day; when the carmine solution should be poured off and the fibrin well washed in water. (Unless the quantity of fibrin placed in the carmine solution has been very large, the latter may serve again.) The fibrin is then ready for use. To preserve it, it is thoroughly pressed so as to squeeze out the water, and then placed in a bottle with a small quantity of ether, which is shaken up with it. By the action of the ether the fibrin becomes somewhat less soluble in gastric juice than when fresh, but it is still readily dissolved. Before the fibrin is used the ether should be washed away with water. When it gives up more than a trace of colour to dilute hydrochloric acid, on warming, it should be thrown away.

"When an experiment is to be made, a small quantity of the stained fibrin should be placed in about five times its volume of HCl (0·02 per cent.) at about 35°C.; in 30 to 60 minutes it will have swollen up; the excess of acid should then be poured off, and equal quantities of fibrin measured out in glass tubes containing exactly 1, 2, or more c.c. as required.

"Having then added the same quantity of fibrin to equal bulks of the acid and pepsin containing extracts, a small difference in the amount of fibrin dissolved, i.e. in the pepsin content of the fluids, is shewn by their different tints. A convenient way of writing down the results for future reference is to note every five minutes which numbers of a series of standard carmine solutions have the same tint as the several digesting mixtures. The digesting mixtures must of course be shaken before they are compared with the standard solutions.

"The standard carmine solutions are thus prepared:

"To 0·1 gram. carmine add from burette 0·1 c.c. of ammonia, mix well, and add 100 c.c. of glycerin. Put into a stoppered bottle and keep in the dark.

"To 6 c.c. of the 0·1 p. c. carmine solution add 54 c.c. of water. To the test tubes add 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 c.c. respectively of the dilute solution by carmine, filling up each test tube to 20 c.c. with water. Thus the test tubes contain respectively 0·1, 0·2.....1 c.c. of the original carmine solution. The colour of these diluted solutions fades with time; this is somewhat delayed, however, by corking the test tubes and keeping them in the dark.

"When extracts which dissolve fibrin rapidly appear to dissolve it at nearly the same rate, they should be diluted and again tested, when a distinct difference in the amount of pepsin may sometimes be ascertained. In these experiments, test tubes of the same diameter must be used, and the conditions, as to temperature and percentage of acid employed, must be the same.

"In order to find out how much more pepsin one extract contains than another, the extract which has been found to contain more pepsin should be diluted until it digests at the same rate as the other extract; the dilution may conveniently be carried out thus:—

"Supposing there are two extracts, of which the one *a* contains more pepsin than the other *b*.

"Take 2 c.c. of $a + 18$ c.c. of dil. HCl (0·2 p. c.), and mix in a beaker: of this put 10 c.c. in test tube (1).

"Add 10 c.c. of the same HCl to fluid in beaker, and of this put 10 c.c. in test tube (2).

"Add 10 c.c. of the same HCl to the fluid in the beaker, and of this put 10 c.c. in test tube (3), and proceed in the same way as far as may be considered necessary.

"Test tubes (1), (2), (3) contain 1 c.c., 0·5 c.c., 0·25 c.c., respectively, of extract *a*.

"Take 2 c.c. of extract $b + 8$ c.c. of dilute HCl (0·2 p. c.). Add to this and to each of the various pepsin solutions made with extracts *a*, 1 c.c. of carmine-stained and already swollen fibrin (which has been previously measured and placed aside in watch glasses), note which of the (*a*) mixtures digests at the same rate as (*b*); if, for example, the (*a*) mixture in test tube (3) digests at the same rate as (*b*), then the original (*a*) extract contains eight times as much pepsin as (*b*).

"2. In comparing the amount of pepsin which can be extracted from equal weights of different stomachs, or of different parts of any one stomach, the mucous membrane should be rapidly washed with salt solution, and the salt solution then sopped up with blotting paper. The stomach should, then, be spread out on glass and the muscular coat removed; the mucous membrane should then be dried first at about 25° C. and then over sulphuric acid. Weighed portions of mucous membrane should then be cut into small pieces and treated with a 2 p. c. HCl, in the proportion of 500 c.c. of the diluted acid for each gramme of dried mucous membrane. Digestion should be allowed to go on at 38° C. for one day (though much the greater part of the pepsin is extracted in two or three hours), the mixture then filtered, and the amount of pepsin in the filtrate determined. If there is any appreciable residue left on the filter, this may again be treated as before with HCl, &c.

"3. Compare the amounts of pepsin that can be extracted from the gastric mucous membrane of guinea-pig or rabbit (1) taken from the fundus, (2) taken from the middle of the greater curvature, (3) taken from the pylorus."

9. *Experiments on Pepsinogen and Pepsin; on Rennet Zymogen and Rennet Ferment.*

The following experiments are taken from Langley's Directions for his Practical Advanced Classes:

(1) "*Preparation of Extracts containing Pepsin and Pepsinogen.* Prepare an aqueous extract of a fresh gastric mucous membrane from a hungry animal.

The stomach should be taken as soon as possible after the death of the animal and washed with solution of Na_2CO_3 (0·01 p.c.), chopped up, placed in a mortar with about 120 c.c. of water, and repeatedly ground. In half-an-hour to an hour the extract may be strained and filtered. Take 50 c.c. of the filtered extract, add 5 c.c. HCl 1 p.c. Warm for half-an-hour, neutralise with x c.c. of 0·2 p.c. solution of Na_2CO_3. Label this Pepsin-extract. Take 50 c.c. of the filtered extract, add to it a mixture of 5 c.c. HCl (1 p.c.) and x c.c. Na_2CO_3. Label this Pepsinogen-extract.

"(2) Effect of Na_2CO_3 on Pepsin.

"Take

	Pepsin extract.		Water.		Sol. of Na_2CO_3 (2 p.c.).	
a.	5 c.c.	+	5 c.c.	+	0	......neutral.
b.	,,	+	4 c.c.	+	·5 c.c.	......·1 p.c. Na_2CO_3.
c.	,,	+	0 c.c.	+	5 c.c.	...... 1 p.c. Na_2CO_3.

"Shake and leave for 5 minutes.

"Mix in three other test-tubes; x being the number of c.c. of HCl required to neutralise 5 c.c. of Na_2CO_3 (2 p.c.).

	Sol. of Na_2CO_3 (2 p.c.)		HCl (1 p.c.)	
a′.	5 c.c.	+	x	fill up each to 15 c.c. with water.
b′.	4·5 c.c.	+	x	
c′.	0	+	x	

"Add *a′*, *b′*, *c′* to *a*, *b*, *c* respectively; each should now be neutral. To 10 c.c. of each mixture add 5 c.c. HCl (·6 p.c.) and 2 c.c. swollen carmine-stained fibrin. Place at 39° C. and observe the rate of solution of the fibrin. In *a* the solution will be rapid, less rapid in *b*, very slow in *c*, shewing that pepsin is rapidly destroyed by sodium carbonate (1 p.c.).

"3. *Pepsinogen.* Repeat the previous experiment, using the pepsinogen extract instead of the pepsin-extract. There will be very little difference in the rates of digestion in *a*, *b*, and *c*, probably it will be rather slower in *c* than in the other two. This may arise either from a slight destruction of pepsinogen, or from a destruction of pepsin formed in preparing the extract, or possibly of pepsin originally present in the gastric mucous membrane.

"4. *Rennet-Ferment.* Use the pepsin-extract prepared in (1). It is best to neutralise the milk employed.

"Take (1) 5 c.c. extract + 5 c.c. of milk
(2) „ „ + 2·5 „ + 2·5 c.c. of water
(3) „ „ + 1 „ + 4 c.c. „

"Place at 39° C. The clot is firmer, and earlier formed the less diluted the milk.

"5. *Rennet-Zymogen.* Use the pepsinogen-extract prepared in 1. With this repeat the experiments described under 4.

"No clotting will take place until the fluid becomes acid from the conversion of milk sugar to lactic acid.

"6. By experiments similar to those given for pepsin, it may be shewn that rennet ferment is rapidly destroyed by Na_2CO_3, and that rennet-zymogen is comparatively slowly destroyed by it."

CHAPTER III.

THE PANCREAS IN ITS RELATION TO THE PANCREATIC JUICE. PANCREATIC DIGESTION.

SECT. 1. INTRODUCTORY OBSERVATIONS CONCERNING SOME POINTS IN THE ANATOMY AND PHYSIOLOGY OF THE PANCREAS.

THE Pancreas is a gland which secretes an alkaline juice and which empties itself into the upper portion of the small intestine.

In addition to this more obvious function, the pancreas plays a remarkable, and as yet incompletely understood, part in relation to the transformations of sugar in the animal economy. The facts which bear on this function will be discussed in a subsequent part of this work.

The Pancreas exists in all air-breathing vertebrates—in mammals, birds, reptiles—and in many, though by no means in all, fishes.

Although it has been usual to say that the pancreas does not exist in invertebrates, it would appear from the recent researches of Krukenberg and others that a glandular organ which is the physiological analogue of the pancreas is widely distributed throughout invertebrates.

The Pancreas is a long narrow gland of a reddish cream colour, which during life varies in tint, being pale when inactive, but turgid and roseate in hue whilst secretion is proceeding. In man the organ lies 'across the posterior wall of the abdomen, behind the stomach and opposite the first lumbar vertebra. Its larger end, the *head*, turned to the right, is embraced by the curvature of the duodenum, whilst its left or narrow extremity, the *tail*, reaches to a somewhat higher level and is in contact with the spleen.'

The normal arrangement is that there exist two pancreatic ducts. One very much larger than the other, *the* pancreatic duct, properly so called, or *Duct of Wirsung*[1], empties itself, in man, into the duodenum between three and four inches below the pylorus by an orifice common to it and to the common bile-duct; the second, very small, *accessory* pancreatic duct, communicates with the first by one or more anastomosing branches and usually has a separate opening into the duodenum. In most animals the chief duct of the pancreas opens into the intestines with, or very near to, the opening of the common bile-duct. In some animals however, as in some monkeys[2], in the ox, the guinea-pig, the rabbit, the principal duct empties itself below the orifice of the bile duct. In the last-named animal the arrangement has been particularly studied by Claude Bernard, who has shewn that whilst the accessory duct usually opens by a common orifice with the bile duct, the principal duct empties into the intestine 35 centimetres below that point[3].

Minute Structure of the Pancreas[4].

The pancreas used to be described as a compound saccular or racemose gland. The observations of Latschenberger[5] and Heidenhain have drawn attention to the fact, however, that the pancreas is more properly a compound tubular gland, i.e. if we follow its branching ducts we find them terminating in blind tubes and not in sacculated recesses.

The gland possesses a capsule of connective tissue whence proceed inwards septa which penetrate the organ and support its constituent lobes and lobules. The interlobular connective tissue supports the blood-vessels, the nerves, and the lymphatics of the gland.

Structure of the ducts of the pancreas.

The pancreas possesses in most animals two, in some more than two, excretory ducts. These ducts are lined by columnar epithelium, which lies upon a basement membrane. On the outer side of this basement membrane, there is no inconsiderable amount of fibrillar connective tissue, and some involuntary muscular fibres. The lobar ducts communicate with the excretory ducts, the former proceeding outwards lead to intralobular ducts, and these again to so-called *intermediary ducts* which communicate directly with the alveoli.

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[1] Wirsung was an anatomist of the 17th century who first observed and delineated the pancreatic duct. He is said to have died by the hands of an assassin in 1643, the same year in which he sent a copy of his engraving of the pancreatic duct to Riolan. Claude Bernard, *Leçons de Physiologie Expérimentale*, Vol. II. (1856), p. 171.

[2] See Milne Edwards, *Leçons sur la Physiologie et l'Anatomie Comparée* (1860), Vol. VI: p. 511.

[3] Claude Bernard, *op. cit.*, pp. 270 and 271.

[4] In his description of the pancreas the author has followed, in several cases almost verbatim, Professor Klein's account in the *Atlas of Histology*, and Professor Heidenhain's in Hermann's *Handbuch* (Vol. v. p. 173), which is based upon his own and Langerhans's observations.

[5] Latschenberger, quoted by Heidenhain, Hermann's *Handbuch*, Vol. v. p. 173.

The epithelium lining lobar and intralobular ducts is composed of short columnar epithelium cells, each with an oval nucleus near the membrana propria on which the cells lie. The epithelium cells become shorter from the lobar towards the *intermediary ducts.* It is to be noted that the epithelial cells of the ducts of the pancreas do not exhibit the 'rod-like fibres' (Klein) which are so clearly seen in the intralobular ducts of the salivary glands.

The intermediary ducts 'are branched canals of various lengths with a small but distinct lumen; each consists of a membrana propria, a continuation of the same membrane of the intralobular duct, lined with a single layer of flattened clear cells more or less elongated, and each with a flattened oval nucleus' (Klein). In some cases, as in the pancreas of the rabbit, these tubes are very long, in others extremely short, the branches of the intralobular ducts appearing to pass almost immediately into the alveoli.

Structure of the alveoli. The secreting cells.

The alveoli which open into the intermediary canals are more or less tortuous tubes composed of a delicate basement membrane which is covered on its inner side by the proper secreting cells which, as Heidenhain aptly remarks, possess specific peculiarities which make it impossible to mistake them for the cells of any other gland. These cells are sometimes described as columnar, but they are not as regular as typical columnar epithelium cells and present much more rounded outlines. The tube is so filled by these cells that a definite continuous lumen is not usually visible.

Appearances of the cells in fasting animals.

The appearances of the pancreatic cells differ greatly according as the gland has been for many hours inactive or long secreting. We shall at present only describe the appearance of the cells of the pancreas of the fasting animal.

Each cell presents, in its fresh living condition, a clear *apparently* homogeneous *outer zone,* directed towards the basement membrane, and a granular *inner zone.* The clear outer zone is relatively small, only forming from one-eighth to one-sixth of the depth of the cell. Carmine stains the outer, clear, zone easily, but scarcely at all the granular inner zone. In many animals, the cells are granular *throughout,* in the fasting state.

The outer zone which in the living cell appears homogeneous is not so in reality, as we learn by the action of perosmic acid, or by maceration for 2 or 3 days in solution of neutral ammonium chromate, which reveal the existence of longitudinal fibrillation.

At the junction of the outer and inner zone of the cells of the fasting pancreas is situated a spherical nucleus which is scarcely, if at all, visible in the living cell, but which is stained by carmine or logwood.

Micro-chemical reactions of the pancreatic cells.

"Water causes the outer zone rapidly to swell up whilst the granules of the inner zone in great part become indistinct.

"Dilute solution of caustic potash or soda (containing only 0·1 per cent.) dissolves the granules almost instantaneously and ultimately the whole cell.

"Dilute acetic and mineral acids of all degrees of concentration render the outer zone so turbid, by causing a granular precipitate, that the distinction between the two zones of the cell vanishes. Glacial acetic acid on the other hand renders the cells clear, merely allowing some fine granules to be perceived, whilst the nuclei come out sharply[1]."

It was remarked by Claude Bernard[2] that the pancreatic secreting cells are dissolved with great ease by bile. This is to be explained by the bile being a liquid which permits tryptic proteolysis to proceed with ease.

Vascular and Nervous Supply of the Pancreas.

Vascular supply.

The pancreas in man receives branches from 1st the hepatic artery, 2nd the splenic artery, and 3rd the superior mesenteric artery; the branches from these arteries form numerous anastomoses. A capillary network surrounds the ultimate acini, but by no means closely, so that often the secreting cells are at a considerable distance from the nearest capillaries.

The veins of the pancreas which run by the side of the arteries empty into the superior mesenteric and into the splenic veins, so that all the blood which leaves the organ has to pass through the liver.

Nervous supply.

In man the nerves of the pancreas are derived primarily from the solar plexus, but for the most part they are immediately derived from the hepatic, mesenteric, or splenic plexuses. They first accompany the arteries, but after reaching the substance of the gland, they follow a separate course. According to Pflüger the fibres of the pancreatic nerves are medullated. Kühne and Lea, and Heidenhain, however, assert that they are non-medullated. Besides nerve fibres, ganglion cells are abundantly scattered through the gland.

SECT. 2. THE SECRETION OF PANCREATIC JUICE.

The secretion of the pancreatic juice is one of those phenomena which it is impossible to study except with the aid of experiments on the lower animals.

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[1] Heidenhain, Hermann's *Handbuch*, Bd. v. Th. 1, s. 175.

[2] Claude Bernard, *Leçons de Phys. Expérim.* Vol. II. p. 486.

Mode of establishing Pancreatic Fistulae.

First experiments of De Graaf.

The first to observe the secretion of the pancreatic juice was De Graaf, who succeeded in making a pancreatic fistula and collecting pancreatic juice[1]. This observer opened the duodenum, inserted a quill into the pancreatic duct, attached a dependent flask to the quill and collected an appreciable quantity of pancreatic juice which he described as clear and somewhat glutinous, and (doubtless following the theoretical views of his master, Franciscus de la Boë Sylvius) he asserted that it had a mixed acid and saline taste.

Many of the older physiologists repeated the experiment of De Graaf, but with comparatively small results.

Claude Bernard's experiments and method.

Claude Bernard was the first to study with care and completeness the flow of the pancreatic juice by the aid of fistulae[2].

The following is Bernard's description of the method which he employed[3]:

"The dog to be experimented upon is placed and firmly held upon its left side, and an incision from seven to eight centimetres long is made in its right hypochondrium, below the borders of the ribs; this incision permits of the duodenum and a part of the pancreas being drawn out. The larger of the two pancreatic ducts is rapidly isolated; in the dog this duct opens obliquely into the duodenum at a point about two centimetres below the common bile duct.

"The pancreatic duct is of a mother-of-pearl colour, and is of the size of a crow's quill; it is seen to be distended by pancreatic juice. The duct is opened with the point of a fine pair of scissors. Immediately, some big drops of a colourless, limpid, pancreatic juice flow away; the juice is viscous, and the viscosity is such that it does not readily mix with the blood which surrounds it, and that it remains isolated, much as an oily liquid or a strong solution of gum.

"A small silver cannula, having approximately a diameter of five millimetres and a length of ten to twelve centimetres, is then introduced into the duct and tied in with a ligature which has previously been placed beneath the duct. The duodenum and pancreas are then returned to the abdomen, and the wound is closed by sutures, care being taken that the free extremity of the silver tube projects. In order to give greater firmness to the arrangement, the cannula is attached to the intestinal wall by means of a single suture, as shewn in the subjoined figure." (See Fig. 13, p. 193.)

A small bladder of caoutchouc is then attached to the cannula so as to collect the juice which is secreted after the operation. The whole operative procedure occupied in Claude Bernard's hands from five to six minutes.

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[1] Regnier de Graaf, 'Tract. Anatom. Med. de succi pancreatici natura et usu,' Lugd. Batav. 1664.

[2] Claude Bernard, *Archives de Médecine, &c.*, Vol. 19 (Jan., 1849) p. 60, *Leçons de Physiologie Expérimentale, &c.*, Paris, 1856, Vol. II. p. 170 et seq., *Leçons sur les Liquides de l'Organisme*, Paris, 1859, Vol. II. p. 337 et seq. Claude Bernard, 'Mémoire sur le pancréas et sur le rôle du suc pancréatique dans les phénomènes digestifs.' *Supplément aux Comptes Rendus de l'Académie des Sciences*, Paris, 1856.

[3] Bernard, *Leçons de Phys. Exp.* Vol. II. p. 180.

Heidenhain's method of establishing a temporary fistula.

The dog to be subjected to operation must be kept without food for 36 hours before the operation, and be deeply narcotized with morphia. An incision is then made in the *linea alba*, midway between the xyphoid process and the umbilicus. The descending part of the duodenum is then drawn out so as to bring into view the adjacent part of the pancreas. In order to find the duct, the following distinguishing character serves:—At the part where the lower lobe of the pancreas recedes from the concave side of the duodenum, there is seen a transparent bridge of mesentery intervening between the intestine and the gland. In this is situated a thick intestinal vein.

On the upper side of this vein the pancreas is directly applied to the intestine; between the vein and the attached part of the pancreas are situated some coarse bundles of vessels. Now, usually, the pancreatic duct runs between the bundles of vessels and the vein; less frequently it is situated between the second and third of the above-mentioned bundles of vessels, whilst in unfavourable cases the bundle is covered by vessels. The length of the duct is here only a few millimetres. By the aid of carbolized ligatures a short glass cannula, having a length of from 6—18 millimetres, is tied in; the cannula has attached to it some thick-walled india-rubber tube. The intestine is provisionally attached to the abdominal wall by two loose ligatures, one being situated above and a second below the duct, the object being to secure adhesion between the intestinal and the abdominal wall. The wound in the abdominal wall is closed, room being merely left for the cannula. The ligatures connected with the intestine are removed after 24 hours, the sutures through the abdominal wall after 36—48 hours. Almost invariably the cannula falls out in a few days.

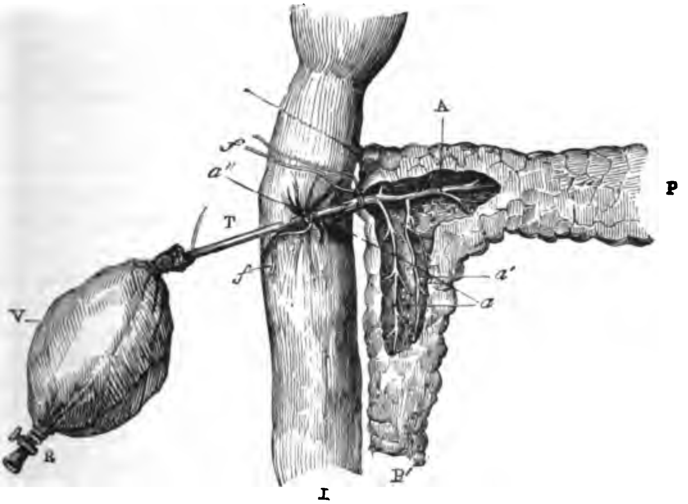

FIG. 13. CANNULA INSERTED INTO THE PANCREATIC DUCT AND ATTACHED TO THE INTESTINAL WALL. (Bernard, see p. 192.)

Method of Ludwig, employed by his pupils Weinmann[1] and Bernstein[2].

'Preference is given to small dogs, as in them the duodenum is more easily reached from the middle line, and is not drawn so far from its natural position by the fistula as in larger animals. The dog must be kept fasting on the day of the operation, as the pancreatic vessels are full during digestion, and bleed easily. Narcotize the animal by injecting opium into the tibial vein, and open the abdomen by an incision about two centimetres long in the linea alba, midway between the ensiform cartilage and the umbilicus. The duodenum is then searched for, and drawn out of the wound along with the attached pancreas, and a thread looped round the duct. Instead of then putting in a cannula, a piece of lead wire is inserted into the duct, so that one end of it passes into the intestine and the other into the gland to a considerable distance. The middle part of it is twisted together, and projects through the wound. Owing to the T shape thus given to the wire, it cannot either slip out or move about in the duct; but wire being chosen which does not fill it up, the flow of the juice is not hindered. Three threads having then been passed through the wall of the duodenum near the duct, the intestine and omentum are replaced in the abdomen, and the duodenum fastened by the threads to the abdominal wall. The wound is then sewed up, care being taken that the twisted part of the lead wire passes through the wound. Twenty-four hours after the operation, the stitches are taken out, but the wire left in. In two or three days afterwards the juice is collected. For this purpose the animal must be supported by straps, which pass under its belly, and are attached to a horizontal bar hung from the roof by a cord and pulley. The dog is then suspended over a table at such a height that it can barely touch it with its toes, in which position it remains perfectly still. A funnel is then attached under the fistula, and the juice collected in a glass below[3].'

Method of Heidenhain for establishing permanent pancreatic fistulæ.

Heidenhain[4] has suggested another method which has enabled him to obtain much more trustworthy results than are furnished by fistulæ established in any of the ways previously described.

The portion of duodenum into which the duct of Wirsung opens is separated from the rest of the intestine by two sections, which are at a distance one from the other of 4—5 centimetres. The continuity of the alimentary canal is re-established as in Thiry's operation by sutures. The isolated cylinder of duodenum is slit longitudinally opposite the entrance of the pancreatic duct, and its mesenteric surface is stitched to the abdominal wall; the wound in the abdominal wall is then brought together. The mucous membrane of the intestine with the papilla of exit of the pancreatic duct is thus brought to the surface of the abdomen, and the secretion may be directly collected.

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[1] Ludwig u. Weinmann, *Ztsch. f. rat. Med.* N. F. Bd. III. (1853) S. 248.

[2] Ludwig u. Bernstein, *Ber. d. sächs. Gesell. d. Wiss. Math. phys. Cl.* 1869, S. 97.

[3] Dr Lauder Brunton, F.R.S., in *Handbook for Physiological Laboratory*, p. 518.

[4] Heidenhain, *Physiologie d. Absonderungsvorgänge.* 4. Abschn. Bauchspeicheldrüse. Hermann's *Handbuch*, Bd. I. Th. 1, S. 179.

Impossibility to obtain all the pancreatic juice secreted.

It has been already said that almost invariably two pancreatic ducts exist. In the dog, the animal which, with rare exceptions[1], has been employed for experiments on the pancreatic juice, this is the case. The larger duct alone can, however, be found in the living animal, and therefore only a part of the pancreatic juice is obtained, the rest always making its way into the duodenum by the accessory duct.

Difficulty in obtaining a continuous normal flow.

The difficulties in obtaining a continuous flow of normal pancreatic juice are extremely great. Usually the juice obtained a few hours, or for a day or two, after the operation possesses the characters which will be referred to hereafter as normal, but very soon, probably as a result of inflammatory changes affecting the gland, the secretion loses its normal characters; it increases in quantity, the percentage of solids diminishing. It often, however, diminishes remarkably in quantity, in consequence, doubtless, of the enlargement of the accessory duct. In the large majority of cases, the cannula soon drops out and, in a very few days, the continuity of the ligatured duct becomes re-established.

GENERAL PHENOMENA OF THE PANCREATIC SECRETION.

The general phenomena of the secretion of pancreatic juice have been discovered by observing, firstly and chiefly, animals in which temporary fistulæ have been established, during the time which elapses before the functions of the gland become, as a result of the operation, perverted; and secondly, animals in which permanent fistulæ have been successfully established; as a rule, for reasons stated already, the fluid obtained from permanent fistulæ soon ceases to be normal.

So long as the condition is perfectly normal, the following is the order of events:

After a fast lasting 24 hours or more, the pancreas ceases to secrete. Immediately after food has been taken, secretion commences, and the rate of secretion increases rapidly, reaching a maximum some time within the first three hours. The secretion then diminishes until a period which Heidenhain states as extending from the fifth to the seventh hour, when a rise occurs, which lasts to 9th—11th hours. The secretion then gradually sinks, until it absolutely ceases; at the 17th hour there is a very scanty secretion; at the 24th hour all secretion has ceased. The fluid secreted in the early periods of digestion is very viscous, and soon gelatinizes on standing; it is highly coagulable. It contains from 6 to 10 per cent. of solid matters. As digestion progresses the juice becomes less viscid, its coagulability diminishes and its solid matters also become less. So that even in the physiological condition we may have a comparatively non-viscid and sparingly coagulable juice.

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[1] Colin of Alfort succeeded in establishing fistulæ in large ruminants, and thus obtained larger quantities of pancreatic juice than any other observer (200—270 grms. per hour in the ox). See 'Expériences sur la sécrétion pancréatique des grands ruminants,' in the *Comptes Rendus*, Vol. XXXII. (1851), and his illustrated descriptions in his *Traité de Physiologie comparée des Animaux Domestiques*, Paris, 1854, Vol. I. p. 631 et seq. Colin (l'Institut, 1851, p. 91, quoted by Donders in his *Physiologie des Menschen*, Vol. I., p. 260) and Frerichs (see 'Verdauung' in Wagner's *Handwörterbuch*), introduced cannulæ into the pancreatic duct of donkeys.

But, in most cases, when a pancreatic fistula has been established matters do not continue as above, and the departure from normality is evidenced, firstly, by the secretion becoming continuous, secondly, by its becoming abundant and non-viscous, as well as by another most important character. The normal juice possesses the power, firstly, of digesting proteids, secondly, of converting starch into dextrins and maltose, thirdly, of emulsionizing and decomposing the neutral fats. Now the non-viscous, abundant, secretion obtained from the majority of cases of permanent fistulæ only possesses the second and third of these properties; it is, that is to say, destitute of, at least very poor in, the proteolytic ferment.

Influence of the nervous system upon the pancreatic secretion[1].

Though the close dependence of the secretion of pancreatic juice upon the various stages of the digestive process must clearly depend upon nervous control, our knowledge of the nervous mechanism is not as complete as might be wished. The following are the principal facts yet ascertained:

1. When the nerves going to the pancreas are all divided, secretion of a diffluent juice is set up and continues; this is analogous to the paralytic saliva which flows after division of all the nerves supplying the submaxillary gland.
2. Electrical stimulation of the medulla oblongata sets up secretion if in abeyance, and increases it if already in process.
3. A useful method, and the only one really at the disposal of the experimenter, for setting up pancreatic secretion in animals with pancreatic fistulæ is to inject a little ether into the stomach[2]: this agent sets up a flow of pancreatic juice which is characteristic of the condition of the gland at the time: thus, if the ether be injected five or six hours after food, there is obtained a flow of viscid and concentrated juice; on the other hand, if it be injected fifteen hours after a meal, it is always diffluent.
4. Secretion is arrested when the act of vomiting is provoked; also by stimulation of the central end of the vagus, and of sensory nerves generally, the arrest in this case lasting long after the stimulation has ceased. The cessation is probably due to a contraction of the blood-vessels of the pancreas.

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[1] The author is indebted for the summary of all the facts which are here stated to Heidenhain's account in Hermann's *Handbuch*, to which the reader is referred, see Vol. v. p. 194 et seq.

[2] Kühne, *Lehrbuch der physiol. Chem.* p. 113.

5. In dogs, atropia stops the secretion of pancreatic juice, but not in rabbits (Pawlow). Pilocarpin induces a sluggish secretion of concentrated juice (Heidenhain). Curare, according to Bernstein, increases the flow of pancreatic juice, but according to Heidenhain generally diminishes it.

Heidenhain's theoretical views as to the nerves of the pancreas.

From the analogy to the salivary glands Heidenhain thinks it likely that in the pancreas as in the salivary glands there exist two classes of secretory nerves which influence activity, viz. truly *secretory*, i.e. which govern the separation of water by the gland, and *trophic*, which by influencing the exchanges of matter in the secreting cells, influence the passage of solid constituents into the secretion.

Circulatory changes in the pancreas.

Bernard pointed out that the fasting pancreas is pale, the active pancreas firm and turgid; and Kühne and Lea have observed the circulatory changes going on in the pancreas of the living rabbit which are referred to in the subjoined paragraph.

Changes in the appearances of the secretory cells which accompany secretion. Concomitant vascular changes.

Our knowledge of the remarkable changes which the secretory cells of the pancreas undergo during digestion is derived first of all from the researches of Heidenhain[1], which have been confirmed by the remarkable observations made by Kühne and Lea[2], who were able to watch the actual process of pancreatic secretion in the case of the transparent pancreas of young rabbits, which was drawn through a small wound in the abdominal wall, and examined under the microscope, special arrangements being employed, which prevented evaporation and cooling. The following is a short but admirable summary by Professor Michael Foster of both Heidenhain's and Kühne and Lea's researches:

'We learn from the researches of Heidenhain that each secreting cell of a pancreas of an animal (dog) which has been fasting for 30 hours or more consists of two zones: an inner zone, next to the lumen of the alveolus, which is studded with fine granules, and a smaller outer zone, which is homogeneous or marked with delicate striæ. Carmine stains the outer zone easily, the inner zone with difficulty. The nucleus, more or less irregular in shape, is placed partly in the one and partly in the other zone. When however the pancreas of an animal in full digestion (about six hours after food and onwards) is examined, the outer homogeneous zone is found to be much wider, the granular inner zone being correspondingly narrower, and in some cases actually disappearing. The whole cell is smaller, and owing to the relatively larger size of the outer zone, stains well. The nucleus is spherical and well formed. If the pancreas be examined at the end of digestion, when its activity has once more ceased, and it has entered into a state of rest, the outer zone is again found to be narrow, the granular inner zone occupying the greater part of the cell, which in consequence stains with difficulty; and the whole cell has once more become larger. There seems to be but one interpretation of these facts. During the time that the pancreas is secreting most rapidly, there is a diminution of the inner zone; that is to say, the inner zone furnishes material for the secretion. But while the inner zone is diminishing, the outer zone is increasing, that is to say, the outer zone is being built up again out of materials brought to it from the blood, though not to such an extent as to prevent the whole cell from becoming smaller. When digestion is ended, after the pancreas has ceased to secrete, the inner zone again enlarges, evidently at the expense of the outer zone, though the latter also continues to increase, causing the whole cell to become bigger. From thence till the next meal, there occurs a partial consumption of the inner zone, so that the outer zone becomes more conspicuous again, though the whole cell becomes smaller. Evidently out of the protoplasm of the cell, which is itself formed at the expense of the blood, the granules are formed, and these being deposited towards the lumen of the alveolus distinguish the outer homogeneous from the inner granular zone, and the secretion is produced at the expense of the granules.

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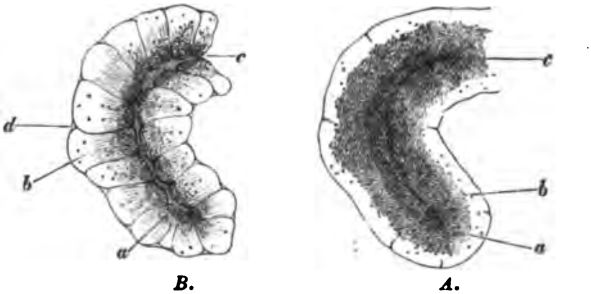
[1] Pflüger's *Archiv*, x. (1875), p. 557.

[2] Kühne u. Lea, 'Beobachtungen über die Absonderung des Pankreas.' *Verhand. d. Naturhist. Med. Vereins zu Heidelberg*, Bd. I. 1877, Heft 5, and *Unters. a. d. physiol. Inst.* Bd. II. S. 448.



B. *A.*

Fig. 14. A portion of the Pancreas of the Rabbit (Kühne and Sheridan Lea), *A* at rest, *B* in a state of activity.

a the inner granular zone, which in *A* is larger, and more closely studded with fine granules, than in *B*, in which the granules are fewer and coarser.

b the outer transparent zone, small in *A*, larger in *B*, and in the latter marked with faint striæ.

c the lumen, very obvious in *B*, but indistinct in *A*.

d an indentation at the junction of two cells, seen in *B*, but not occurring in *A*.

'Kühne and Sheridan Lea[1], observing, under the microscope, the pancreas of the living rabbit, have been able to watch the actual process of secretion; and their results, while they extend, in the main corroborate those of Heidenhain. In the quiescent pancreas of the rabbit, Fig. 13 A, the cells are for the most part filled with granules, the transparent outer zone being reduced to small dimensions; the outlines of the individual cells are very indistinct, with the margins of the alveoli smooth; the lumen of the alveolus is obscure; and the blood supply is scanty. Upon secretion being set up, Fig. 13 B, the margins of the active alveoli become indented through a bulging of their constituent cells, the outlines of which now become distinct; the granules retreat towards the inner zone, bordering on the cavity of the alveolus, and as secretion goes on, evidently diminish in number, the whole cell becoming hyaline and transparent from the outer border inwards; at the same time the blood-vessels dilate largely, and the stream of blood through the capillaries becomes full and rapid.'

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[1] *Verhandl. Naturhist. Med. Vereins*, Heidelberg, Bd. I. (1877), Heft 5, and Bd. II. (1882), Heft 4.

Quantity of pancreatic juice secreted.

Allusion has already been made to the difficulties which attend attempts to collect the whole of the pancreatic juice secreted by an animal; these difficulties explain the discrepancies in the statements of various observers concerning the amount of juice secreted either during a single act of digestion or in a given time, as in 24 hours. As a result of observations on temporary fistulæ, it has been estimated that, assuming the rate of secretion in man to be in proportion to that in the dog, a man would secrete in 24 hours from 211 to 347 grammes[1]. The older estimates founded on the observations of Ludwig and Weinmann were much higher[2].

SECTION 3. THE PHYSICAL AND CHEMICAL CHARACTERS OF THE PANCREATIC JUICE.

In describing the general phenomena of the pancreatic secretion some of its more prominent physical and chemical characters have been referred to, though a complete description has been reserved for this section.

Physical Characters.

The juice obtained from temporary fistulæ or in permanent fistulæ when changes in the gland have not occurred, is, as has already been said, a more or less viscid, gluey liquid.

It contains suspended in it constantly certain morphological elements (Kühne[3]). These are:—colourless blood corpuscles of the smaller kind, which exhibit sluggish, yet perceptible, amœboid movements: corpuscles which are larger than the above-mentioned colourless corpuscles, but smaller than the so-called salivary corpuscles of mixed saliva with which, however, they agree in all other particulars. These corpuscles have in their interior granules which exhibit lively Brownian movements and possess one to four nuclei. At favourable temperatures the morphological elements are digested and dissolved.

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[1] Kühne, '*Lehrbuch der physiologischen Chemie*, 1866, p. 114.

[2] The reader is referred for data relating to the older experiments to Donders, *Physiologie des Menschen*, Vol. I. p. 263.

[3] Kühne, 'Ueber das Secret des Pankreas,' *Verhand. d. Naturhist. Med. Vereins zu Heidelberg*, Bd. I. Heft 4.

Coagulation. Claude Bernard described the pancreatic juice as becoming more viscid as it cooled. Kühne has however found that when cooled (as to 0° C.) it undergoes a true coagulation, separating into a gelatinous and a diffluent part. In consequence of this property, the pancreatic juice often forms compact opaque clots in silver cannulæ.

Alkalinity. The pancreatic juice is invariably alkaline.

Taste. The pancreatic juice possesses a saltish taste.

Specific gravity. The fluid of the temporary fistulæ has a higher specific gravity than that of even successful permanent fistulæ. The former has a specific gravity of 1030, the latter between 1010 and 1011[1].

General Chemical Characters.

When heated on the water bath to 75° C., pancreatic juice, obtained from a temporary fistula, is coagulated so completely as to become converted into a white opaque mass, from which there separates a slightly opalescent fluid more alkaline than the uncoagulated juice, which is precipitated by acetic acid and contains alkaline albuminate.

When pancreatic juice is dropped into water, the drops coagulate as they fall, the precipitate being soluble in NaCl and dilute acids. When dropped into very dilute acids a similar coagulation takes place, but the coagula are dissolved when shaken up with the acid.

Alcohol added to pancreatic juice produces an abundant white flocculent precipitate, which, even when washed with or digested in absolute alcohol, is for the most part soluble in water at 0° C. Acetic acid does not precipitate this watery solution; after being acted upon for some time by acetic acid, on neutralization a proteid precipitate is obtained. The portion of the alcohol precipitate which is insoluble in water behaves as a coagulated albumin.

The alcoholic precipitate referred to carries down with it the various ferments whose action will be described in the sequel. The pancreatic juice is precipitated by the concentrated mineral acids, by metallic salts, by tannic acid. Chlorine or bromine water added to fresh pancreatic juice occasions a white precipitate. If however this reagent be added to pancreatic juice which has been exposed to warmth for some time, it occasions a red colour (Tiedemann and Gmelin).

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[1] Maly, see 'Pankreassaft,' in Hermann's *Handbuch*, Vol. v. part 1, p. 187. The author does not know the original sources whence these data have been obtained and does not hold himself responsible for their accuracy.

Pancreatic juice undergoes putrefaction with the utmost ease. The red colour above referred to as brought about by chlorine is due to a body yet unknown which results from decomposition (Tryptophan). In a juice which exhibits the chlorine reaction, decomposition rapidly proceeds a step further, and then the reaction no longer occurs; on, however, adding impure coloured nitric acid to the now foul-smelling liquid, a red colour is developed which is due to indol (C_8H_7N).

Presence of three enzymes in the pancreatic juice.

Normal pancreatic juice contains at least three distinct enzymes, which will be treated of at length in the sequel. These are: 1, a proteolytic ferment, which at suitable temperatures and in solutions which are neutral and faintly alkaline, readily decomposes proteids with the production of peptones and amido-acids, such as leucine and tyrosine: 2, a diastatic ferment, similar to that which exists in saliva, converting starches into erythrodextrins, achroodextrins and maltose: 3, a fat-decomposing ferment which brings about the hydrolytic decomposition of the neutral fats into glycerine and fatty acids. Although these three ferments always co-exist in normal pancreatic juice, in the continuous thin secretion from permanent fistulæ, the second and third ferments are sometimes found unaccompanied by the first or proteolytic enzyme.

Are leucine and tyrosine constituents of the pancreatic juice?

In 500 c.c. of freshly secreted pancreatic juice obtained from a large number of dogs, Kühne[1] was unable to discover a trace of tyrosine. Leucine was present, but in so small a quantity as to be only discoverable by the microscope.

Percentage composition of perfectly normal pancreatic juice.

The viscous secretion obtained from recently established fistulæ (dog) contains approximately in 1000 parts

900 parts of water,
90 „ organic solid matter,
10 „ inorganic salts.

The organic solid matter is composed mainly of proteids and ferments. Generally the more abundant the flow, the smaller the amount of solid matter in solution. The salts consist mainly (that is to the extent of about seven-tenths) of sodium chloride; the remaining salts are sodium carbonate, with traces of sodium phosphate, earthy phosphates and traces of iron. In the first of the analyses given in the subjoined tabular view, Schmidt found the inorganic matters per 1000 to be 8·8, and in this the NaCl amounted to 7·35.

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[1] Kühne, 'Ueber das Sekret des Pankreas,' *loc. cit.*

Percentage composition of the thin juice of permanent fistulæ.

The thin juice secreted continuously by permanent fistulæ is sometimes not coagulable by heat alone, but requires the addition of an acid. It contains from 10—20 parts per 1000 of solid matters.

COMPOSITION OF PANCREATIC JUICE (C. SCHMIDT[1]).

	I.		II.		
	From temporary fistulæ.		From permanent fistulæ.		
	a	*b*	*a*	*b*	*c*
Water in 1000 parts	900·8	884·4	976·8	979·9	984·6
Solids " "	99·2	115·6	23·2	20·1	15·4
containing					
Organic matters	90·4	—	16·4	12·4	9·2
Inorganic matters	8·8	—	6·8	7·5	6·1

SECTION 4. THE PANCREATIC ENZYMES CONSIDERED IN DETAIL.

In the last section in discussing the general chemical composition of the pancreatic juice we have referred to the fact that it possesses very remarkable properties of acting on organic bodies, and that these are supposed to be dependent upon the existence in the juice of three distinct enzymes. It has further been stated that when the pancreatic juice is precipitated by alcohol, the precipitate which falls carries down with it the ferments. The precipitated body was indeed formerly supposed to constitute the ferment, and the opinion prevailed that this ferment is possessed of various properties. We now know, however, that the activity of the so-called *pancreatin*, is due to a mechanical entanglement of the ferments, which apparently are not associated with one body but are distinct bodies.

We must now in the first place carefully examine the chief facts relating to each of the ferment actions of the pancreatic juice, and study the products which take their rise in these.

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[1] This table, which is compiled from Schmidt's observations, is reprinted from Maly, *op. cit.*, Hermann's *Handbuch*, Vol. v. a, p. 189.

I. *THE DIASTATIC ENZYME.*

The saliva, we have seen, is a liquid which only possesses an amylolytic action in a few animals, the great majority of animals having a saliva which possesses no diastatic ferment.

History of the discovery.

Bouchardat and Sandras[1], in 1845, first discovered and established with precision by means of observations carried on with the aid of pancreatic infusions, as well as of small quantities of pancreatic juice obtained from hens and geese, that this secretion possesses powerful diastatic properties, starch being rapidly converted into glucose. They extended their observations to rabbits.

It has been erroneously stated by many writers possessing high authority that this discovery was first of all made by Professor Valentin of Bern. The statement, so far as the author can make out, first appeared in Frerichs' article 'Verdauung,' in Wagner's *Handwörterbuch der Physiologie,* Vol. III. part 1, p. 847, and has been repeated by subsequent authors (Donders, Colin and Maly). Thus Donders says (*Physiologie des Menschen,* Vol. I. p. 264), "Valentin scheint zuerst gefunden zu haben, dass der Bauchspeichel die Eigenschaft besitzt Stärkemehl schnell in Zucker umzuwandeln, und alle späteren Untersuchungen haben dies bestätigt."

The observations of the distinguished Bernese physiologist Valentin are recorded in his *Lehrbuch der Physiologie* (Braunschweig, 1844), Vol. I. p. 340 and 341. They were experiments in which a complex mixture of alimentary substances and of digestive juices were observed, and so far from proving the conversion of starch into sugar, scarcely warranted even the modest and cautious conclusion which Valentin expressed as follows: "Wie man sieht, erlauben diese Erfahrungen noch keine irgend bestimmenden Schlüsse. Höchstens deuten sie darauf hin, dass vielleicht die Pancreasflüssigkeit die Fähigkeit habe, die Stärke löslich zu machen und bisweilen eine Umsetzung derselben einzuleiten." No better example could be given of the way in which errors in scientific history have been propagated by the habit of quoting opinions at second hand without taking the trouble to examine the evidence on which the opinions were based!

The action of pancreatic juice on raw starch is generally stated to be but slight[2]; on starch mucilage it is surprisingly great. At 35° the action is intensely energetic.

The diastatic action of the diffluent, abnormal, secretion from permanent fistulæ is said (Kühne) to be as powerful as that of the coherent concentrated liquid of permanent fistulæ.

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[1] Bouchardat et Sandras, 'Des fonctions du pancréas.' *Comptes Rendus, de l'Académie des Sciences,* Vol. XX. 14 Avril, 1845.

[2] Kühne is not of this opinion. 'Aus roher Stärke, wie aus gekochter bildet ein einziger Tropfen des Secrets mit rapider Geschwindigkeit Zucker.' *Lehrbuch,* p. 117.

The diastatic enzyme is not only contained in the pancreatic juice, but likewise in the tissue of the pancreas.

An infusion of the pancreas acts upon starch exactly in the same manner though not so energetically as the pancreatic juice, and we may therefore employ in our experiments on the diastatic enzyme of the pancreas such an infusion, instead of pancreatic juice.

Preparation of active Solutions containing the Enzymes of the Pancreas.

It is exceedingly convenient to have at our disposal permanent solutions of the enzymes of the pancreas. The fat-splitting ferment cannot, however, be indefinitely kept and special precautions must be observed in order to extract it. See page 210.

The pancreatic enzymes soluble in glycerin.

All the pancreatic enzymes are extracted by glycerin from the gland, and such a glycerin solution may be preserved conveniently.

Solubility of the enzymes in chloroform water.

Roberts was the first to point out that they are likewise soluble in a saturated aqueous solution of chloroform, and the solution keeps very well (Roberts[1]). The presence of chloroform interferes, however, with the operation of testing for sugar by Fehling's solution. Salkowski[2] many years after Roberts recommended the use of chloroform as a solvent of enzymes.

Solubility of the enzymes in a solution containing boracic acid and borax.

Roberts[3] has found that for experimental purposes a good and lasting extract of the pancreas may be made by extracting the organ with a solution which contains 'three or four per cent. of a mixture of two parts of boracic acid and one part of borax.'

Preparation of a brine extract of the pancreatic enzymes (Roberts).

When the fresh pancreatic tissue is comminuted and placed in a saturated solution of common salt, the pancreatic enzymes are dissolved and powerfully active solutions of these (the fat-decomposing ferment alone excepted) are obtained. This method of extracting the pancreatic enzymes has been strongly recommended by Harris and Gow[4].

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[1] Roberts, 'On the Digestive Ferments,' &c. *The Lumleian Lectures for* 1880. London, 1880, p. 26.

[2] Salkowski, 'Ueber das eiweisslösende Ferment der Fäulnissbakterien.' *Zeitschrift für Biologie*, Vol. xxv. (1889), p. 92.

[3] Roberts, *op. cit.* p. 19.

[4] Harris and Gow, 'Ferment Actions of the Pancreas in different Animals.' By Vincent D. Harris, M.D., F.R.C.P. and William J. Gow, M.D., M.R.C.P., *Journal of Physiology* (Oct. 1892), Vol. 13, pp. 469—492.

Kühne's method of preparing and preserving the pancreatic tissue.

When discussing the methods of preparing active solutions of Trypsin, a method will be described whereby the pancreatic tissue may be indefinitely preserved, and active extracts obtained at any time. The method consists essentially in dehydrating the finely divided pancreas by macerating it in alcohol, and afterwards extracting with boiling ether. The insoluble residue is exposed to the aïr, so as to allow the ether to evaporate, when there is left a white friable solid mass, which may be powdered and kept in a stoppered bottle for future use[1].

By digesting one part of dried pancreas at 40° C. for 3, 4 or 5 hours in 10 parts of a 0·1 solution of salicylic acid, extracts of great activity may be obtained.

Preparation of a solution of the pancreatic enzymes in dilute alcohol (Roberts).

One of the best methods of preparing a very active solution of the pancreatic enzymes is the following, in which advantage is taken of the fact that they are very soluble in water and that their aqueous solutions are preserved from decomposition by a small addition of alcohol:—

Digest fresh pancreas freed from fat, and then chopped up, in four times its weight of dilute alcohol, containing 25 per cent. of rectified spirit (i.e. of alcohol of sp. gr. 0·838). The digestion is continued for four or five days with occasional agitation. The mixture is then filtered through paper. Filtration is much facilitated by the addition to the solution of 0·02 per cent. of acetic acid (containing 28 per cent. of the anhydrous acid).

Degree of Activity and Mode of Action of a Solution of the Diastatic Ferment.

Following the last method, a solution may be obtained possessed of remarkable activity, though this differs according to the animal employed and its individual circumstances &c. The pig yields the most active solution, its diastatic value being more than ten times as great as that prepared from the pancreas of the ox or sheep.

Nature of the action exerted by the diastatic ferment of the pancreas.

The action of the diastatic ferment of the pancreas and pancreatic juice appears in essential particulars to resemble that of the saliva and salivary glands; i.e. the products formed are the same, the conditions of activity are similar, &c. According to v. Mering and Musculus[2] in both cases there are formed achroodextrins, maltose and a little grape-sugar.

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[1] Pancreas thus prepared may be obtained under the designation 'Pankreas, trocken nach Kühne,' from Dr Grübler, Bayersche Strasse 12, Leipzig, and is sold at the price of 7*s.* per 100 grammes.

[2] v. Mering u. Musculus, 'Ueber die Umwandlung von Stärke und Glycogen durch Diastas, Speichel, Pankreas und Leberferment.' *Zeitschr. f. phys. Chem.* Vol. II. (1878—79), p. 403.

Temperature at which diastatic enzyme of pancreas is most active.

Roberts has found that the action of pancreatic diastase on starch mucilage increases in speed from zero to 30° C. From this to 45° C. the rate of action continues steady. Above 45° the action becomes slower and slower, and ceases between 60° and 70°.

Rapidity of action of a solution containing the diastatic ferment influenced by the amount of ferment.

We have seen that within a certain range of temperature the rapidity of the action upon starch increases. Temperature and all other conditions being exactly similar, the rapidity of the action will depend upon the quantity of enzyme present. This is well brought out in the following remarks[1].

'The speed at which a given quantity of starch is transformed by diastase depends essentially on the proportion of ferment brought to act upon it. In the above experiments (experiments in which a minimal quantity of diastatic solution acted upon starch) the proportion of diastase was very minute in comparison with the amount of starch, and the action went on slowly for forty-eight hours. But if we reverse these proportions and mix a small amount of starch with a large amount of diastase the transformation is instantaneously accomplished. If a test-tube be half filled with an active extract of pancreas and a few drops of starch mucilage be quickly shaken therewith, you cannot detect the reaction of starch or dextrine in the mixture, however prompt you may be with the testing—the transformation has followed on the admixture as instantaneously as the explosion of the charge follows the fall of the trigger. Between these extremes there are all gradations.'

Estimate of the activity of the diastatic ferment.

Roberts has estimated that pancreatic diastase is able to transform into sugar and dextrin no less than 40,000 times its own weight of sugar[2].

Estimate of the amount of ferment present in the pancreatic tissue of the pig, ox and sheep.

Roberts has compared by his method of diastasimetry (see chapter I. p. 56) the diastatic activity of the tissue of the pancreas of the ox, sheep, and pig, and he finds that the ferment contained in 1 gramme of the fresh pancreas of the pig can convert 5 grammes of dry starch into products which give no colour reaction with iodine; the same weight of the pancreas of an ox would only act in a similar manner upon 0·4 grm. of starch; and the same weight of the pancreas of the sheep would act upon 0·44 grm. of starch.

Is there a Zymogen of the Diastatic Ferment?

In giving the history of the proteolytic ferment of the pancreas—trypsin—abundant anatomical and experimental evidence will be adduced to prove that there exists in the secreting cells of the pancreas a body from which the ferment is derived; this body from which the ferment is liberated, perhaps by a process of dissociation, may be extracted from the pancreas, and solutions of it are found not to possess proteolytic properties, though they may acquire these by the action of certain external agents. This antecedent of the ferment has been termed by Heidenhain, *Zymogen*. In discussing the origin of pepsin we have already referred to evidence which appears to shew that in the case of that enzyme also there probably exists an antecedent (which some have even named Pepsinogen) which does not possess proteolytic properties but which acquires these, for example on treatment with acids. We have also adduced facts which prove the existence of a zymogen of the rennet-ferment.

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[1] Roberts, *Lumleian Lectures*, page 40.
[2] Roberts, *op. cit.* p. 39.

It is probable that what is certainly true of the proteolytic ferment of the pancreas may be true of its diastatic ferment, and we may ask ourselves, Is there any evidence in support of an antecedent or zymogen of this ferment?

The experiments of Liversidge point to the existence of a zymogen of the pancreatic diastatic enzyme.

Two years before Heidenhain[1] published the remarkable paper, rich in fresh facts, in which he announced his discovery of the zymogen of the proteolytic ferment, Liversidge, working in Foster's laboratory, had published facts, which have not received the notice which they deserve, and which point, as the Author thinks, to the probable existence in the pancreas of an antecedent of the diastatic ferment.

Liversidge removed the diastatic ferment from the pancreas by long-continued washing in water. The minced pancreas which had been thus exhausted was transferred to a filter and allowed to remain exposed to the air for a few hours, when, on again treating it with a small quantity of distilled water, a very active diastatic solution was obtained. Again, he shewed that in order to exhaust minced pancreas of its diastatic ferment by the action of glycerin, contact of large quantities of glycerin during fourteen months was necessary. The pancreatic tissue which had been rendered thus inactive, after standing on a muslin filter for six hours, readily gave, not only an active aqueous extract, but also yielded an active glycerin solution[2].

At the same time there are two points in connection with this question which should be borne in mind. (1) In the above experiments it can hardly be regarded as absolutely certain that the diastatic action exerted by the pancreas after exposure was not due to bacteria. (2) The zymogens of trypsin, pepsin, and of the rennet-ferment are all soluble in water (or water containing a little salt); in an aqueous extract, the zymogen can be shewn to be present, if when treated in certain ways the solution shews ferment activity.

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[1] Heidenhain, 'Beiträge zur Kenntniss des Pankreas,' Pflüger's *Archiv*, Vol. x. (1875), p. 557. The part containing this paper was published on June 25th.

[2] Liversidge, 'On the Amylolytic Ferment of the Pancreas' (from the Physiological Laboratory in the University of Cambridge), *Journal of Anatomy and Physiology*, Vol. VIII. (1874), p. 23. The number of the Journal containing this paper was published in November, 1873.

Now it may be argued that the aqueous extract of the pancreas or of the salivary glands contains no diastatic zymogen for, so far as is known, there is no treatment which will increase its diastatic activity. If we could obtain an extract of the pancreas which when added to starch exerted no effect on it, but which when treated with a little acid and neutralized, or by some similar method, rapidly converted starch to sugar, then we should have presumptive evidence of the existence of a diastatic zymogen. From the facts in our possession we appear compelled to admit either that a zymogen of the diastatic enzyme does not exist, or if it exists that it differs from the zymogens of analogous ferments by its insolubility in water.

The grounds for believing in the independence of the diastatic enzyme.

The individuality of the diastatic enzyme, and especially its independence of the one concerning which our knowledge is most definite, viz. trypsin, is proved by the following considerations:—

1. A pancreatic extract, or pancreatic juice, may be obtained, which is rich in the diastatic ferment and contains no proteolytic ferment. This, as already previously stated, is often the case with the secretion obtained from so-called permanent pancreatic fistulæ.

2. In different animals not only the absolute but the relative richness in diastatic and proteolytic enzymes differs. Thus Roberts, as we have already stated, found that the diastatic activity of the pig's pancreas is more than ten times as great as that of the sheep. The proteolytic activity of the pancreas of the sheep is, on the other hand, considerably greater than that of the pig.

3. As will be pointed out in the sequel, although attempts to obtain the pure ferments have hitherto not been successful, methods are known by which one ferment may be obtained absolutely free from the others.

Attempts to isolate the Diastatic Ferment.

First experiments of Bouchardat and Sandras.

It was Bouchardat and Sandras who first attempted to separate the agent which conferred upon the pancreatic juice the property which they had discovered, of converting starch into sugar. They treated infusions of pancreas with water, and precipitated the solution with alcohol. The precipitate they found to be again soluble in water and to possess powerful diastatic action. They termed it *pancreatine.* The body thus precipitated must, however, as we know, have consisted of a mixture of the several pancreatic ferments.

Danilewski's method[1].

The first to attempt to separate the diastatic ferment was Danilewski. The principle of his method was to precipitate aqueous infusions of pancreas, which had been treated with magnesium carbonate, with collodion, which carries down with it proteids and the proteolytic ferment in a gelatinous form. The filtrate from this precipitate is concentrated *in vacuo* and treated with strong alcohol, which throws down a flocculent precipitate. This is digested in a mixture of equal parts of alcohol and water, which dissolves the diastatic ferment, a little tyrosine and some salts, and leaves some albumin undissolved. The liquid is dialyzed, concentrated *in vacuo* and precipitated by absolute alcohol. The body thus thrown down possesses in a feeble degree proteolytic properties, due to remaining traces of the proteolytic ferment, but in an intense degree diastatic properties. It does not exhibit proteid reactions, i.e. both the xanthoproteic and Millon's reaction fail.

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[1] Danilewski, 'Ueber specifisch wirkende Körper des natürlichen und künstlichen pancreatischen Saftes,' Virchow's *Archiv*, Vol. xxv. p. 279.

Cohnheim's method[1]. Cohnheim obtained from an infusion of pancreas, by the method which he had already employed in the separation of salivary diastase (see p. 38), a ferment which did not manifest proteid reactions, and which was as active as the body which he had prepared from saliva; it was entirely free from proteolytic action.

v. Wittich's method[2]. Finely divided pancreas is dehydrated, first by being placed in strong alcohol, and afterwards in absolute alcohol, the action of which should be continued for some time. The dry solid separated from the alcohol is then macerated in glycerin. The glycerin solution is then precipitated with alcohol. The precipitate is dried at a low temperature. It is partially soluble in distilled water.

The further purification may be carried on as follows[3]:

The precipitate produced by alcohol in the first glycerin solution is washed with strong spirit, and after partial drying, by the spontaneous evaporation of the spirit, is again treated with glycerin, and this second glycerin extract in its turn precipitated with spirit. There is thus obtained a body soluble in water, possessed of great diastatic power, and, according to v. Wittich, destitute of the proteolytic power. According to this author, when glycerin acts upon pancreas directly, it extracts both the proteolytic and the amylolytic ferments, but when the gland tissue has been first thoroughly dehydrated only the second of these. Hüfner[4], however, controverted this statement, and by following v. Wittich's method he obtained a body which possessed all three of the ferment actions which are characteristic of the pancreatic juice. It is to be remarked that, according to Kühne, pure trypsin is not soluble in glycerin, a fact which makes it likely that the discrepancy between v. Wittich's and Hüfner's results may have depended upon the former having eliminated all traces of water in his preparation, whilst the latter may not have done so.

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[1] Cohnheim, 'Zur Kenntniss der zuckerbildenden Fermente,' Virchow's *Archiv*, Vol. XXVIII. p. 251.

[2] v. Wittich, Pflüger's *Archiv*, Vol. II.

[3] Liversidge, *op. cit.*, p. 24.

[4] Hüfner, 'Untersuchungen über die ungeformten Fermente,' *Journ. für prakt. Chemie*, N.F., Vol. V., p. 372.

The Chemical Composition of the Diastatic Enzyme.

On this matter our information is such that we can say little, and that consists mainly of negative assertions.

From the observations of Cohnheim and of Liversidge it results that the diastatic ferment does not give the reactions of the proteid bodies. Hüfner's discrepant results are accounted for by the fact that his *pancreatin* did contain considerable quantities of trypsin.

The diastatic ferment is a nitrogenous body, which possesses a composition widely different from that of the proteids.

The analysis of the ferment prepared by Liversidge, and which strangely only furnishes the amount of carbon and nitrogen, gave the following results:

Carbon	per cent.	34·925
Nitrogen..................	„	11·020

Hüfner found his so-called Pancreatin to have the following composition:

Carbon	40·9
Hydrogen	6·85
Nitrogen	13·64

This analysis agrees with the hypothesis that Hüfner's pancreatin consisted of a mixture of diastatic ferment and proteids, a hypothesis of which he has besides furnished the proofs in the account of his experiments.

SECT. 5. THE FAT-DECOMPOSING ENZYME.

The Researches of Claude Bernard.

Eberle[1] announced in 1834 that a watery infusion of the pancreas, when shaken with oil, emulsionized it, a creamy emulsion being obtained, and he was led to surmise that one of the functions of the pancreatic juice consists in emulsifying fats and thus favouring their absorption by the lacteals.

This older observation has generally been forgotten, and the whole credit of suggesting as well as of elucidating the part played by the pancreatic juice in the digestion and absorption of fats ascribed to Claude Bernard.

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[1] Eberle, *Physiologie der Verdauung*, Würzburg, 1834.

It was in the year 1846 that Claude Bernard, being engaged in a comparative study of the process of digestion in carnivorous and herbivorous animals, was struck by the fact that when dogs were fed upon fatty matter this appeared to undergo a modification almost as soon as it passed into the small intestine, whilst when rabbits were similarly fed the change occurred somewhat further from the pylorus. Again, Bernard observed that after a fatty diet the lacteals of dogs were filled with white opalescent chyle from the pylorus downwards, whilst in rabbits the lacteals near the pylorus did not contain white chyle, while those situated lower down did. Bernard then discovered that this difference in the appearance and absorption of fatty matters coincided with the difference in the situation at which the pancreatic ducts join the small intestine in the dog and rabbit respectively. In the dog the principal duct empties itself, together with the bile duct, into the duodenum very near to the pylorus; whilst in the rabbit the principal duct joins the small intestine from 30 to 35 centimetres (12 to 14 inches) below the point of entrance of the bile duct.

When this relationship had been found to exist between the situation at which the pancreatic juice is poured into the intestine and the situation where fat begins to be modified, it was natural to inquire whether the juice was not the active agent in effecting the modification of fatty matter, and in causing the appearance of milky chyle in the lacteals, and as a result of his investigations Claude Bernard was led to the discovery of the facts about to be commented upon[1].

The pancreatic juice possesses the power of emulsionizing fats.

Oil or fatty matters which are fluid at the temperature of the animal body are very readily emulsionized by the pancreatic juice.

If two grammes of alkaline and viscous pancreatic juice be shaken up in a test-tube with one gramme of olive oil, almost instantly, a perfect emulsion is obtained, the liquid resembling milk or chyle; the same result is obtained if for olive oil we substitute fats, such as butter or mutton suet, which melt at a temperature below 40° C. Temperature appears to have considerable influence in the process. Thus, when one gramme of lard is agitated with two grammes of fresh, normal pancreatic juice, the process of emulsionizing commences even in the cold, but when the temperature is raised to 35° or 38°, a white creamy emulsion is obtained instantly[2].

Emulsions obtained in this way are remarkably persistent, and, according to Kühne[3], the fat in them exists in even a finer state of division than in milk.

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[1] This short account of the way in which Claude Bernard was led to investigate the action of the pancreas is taken from his *Leçons de Physiologie Expérimentale*, Vol. II. pp. 178 and 179.

[2] Bernard, *Leçons* (1856), Vol. II. p. 256.

[3] Kühne, *Lehrbuch*, p. 122.

Upon what does the emulsionizing power depend?

Claude Bernard was led to believe that the property of emulsionizing fats which the pancreatic juice possesses in so extraordinary a degree, depended upon a ferment, which at the same time occasioned the remarkable change to be immediately referred to, and which he termed the '*ferment émulsif.*' In this view Bernard was wrong. It is apparently only in an indirect way that a ferment leads to the emulsionizing of the fats.

Brücke has shewn that when an oil or a fat which contains a mere trace of free acid is shaken with a weak solution of carbonate of sodium an emulsion is readily obtained, whilst if the oil be perfectly neutral no such emulsion is obtained. It will be shewn that at the temperature of the body the pancreatic juice does lead to the acidification of fats; as the juice moreover contains carbonate of sodium, the conditions readily arise which are required for the production of an emulsion. It is remarked by Kühne, and with justice, that probably the proteid matters in the pancreatic juice play an important part in the emulsionising action[1].

Roberts states that he has been unable to obtain any extract of pancreas which possessed any special power of emulsifying fats, but in this respect he differs from other trustworthy observers who have asserted that watery infusions of pancreas do possess such power, and is unquestionably in error.

The Pancreatic Juice decomposes the Neutral Fats.

Bernard discovered that when emulsions are made by mixing fresh, alkaline pancreatic juice with a neutral fat, such as olive oil or lard, and the emulsions are maintained at the temperature of the animal body, an acid reaction is very soon developed. The observation has been confirmed again and again, by Berthelot amongst others.

Claude Bernard had found that when butter is kept at the temperature of the body with pancreatic juice, the odour of butyric acid is soon perceived.

Berthelot tried the experiment with synthetically prepared monobutyrin and, by the action of pancreatic juice upon it, obtained besides undecomposed monobutyrin, a mixture of free glycerin, butyric acid and a soap.

According to Bernard, pancreatic tissue also decomposes fats.

The property which the pancreatic juice possesses of decomposing the neutral fats is shared by the pancreatic tissue itself; it is indeed laid down by Claude Bernard as the characteristic of this tissue that it possesses the property of *instantaneously* decomposing butyrin.

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[1] Kühne, *Lehrbuch*, p. 122.

Various methods have been suggested by Claude Bernard for exhibiting this characteristic reaction, of which the following are the chief; the second being specially recommended.

1. An infusion of linseed is shaken with a little butter so as to emulsionize it completely, and is then coloured faintly blue by means of litmus. If to a little of the blue emulsion a fragment of pancreas be added, and the mixture be digested at 38° C. for some time the blue colour changes to red.

2. An ethereal solution of butter (which should be neutral) is made, and a solution of litmus of such strength that a stratum half a millimetre thick presents a distinctly blue tint.

A fragment of pancreas is placed on a glass slide, and is then treated with a drop of rectified spirit; it is then teased with needles, so that the alcohol should bathe every part. Enough alcohol should be in contact with the tissue to allow of its remaining bathed in it for about a quarter of an hour. At the end of that time, the excess of alcohol is sucked up with blotting-paper, and the fragment of tissue is irrigated with a drop or two of the ethereal solution of butter, and is teased at the same time, so as to bring the fatty matters as much as possible in contact with the tissue. A fragment of the tissue is then placed in a glass cell, one millimetre deep, containing a tincture of blue litmus, which is then covered with a cover-glass. In a few moments, as the tissue becomes soaked with the solution of litmus, a red area appears around it, and after a certain time the whole of the liquid becomes red, the red colour being intense in proportion as the tincture of litmus was blue.

It may be asked whether the acid reaction is not due to the tissue of the pancreas? but that it is not appears to be proved by the fact that when the pancreas is treated exactly as stated above, with the exception that no fat is added, the acid reaction is not developed. The Author can, from his own observations, confirm these statements of Bernard.

The fat-decomposing power of the pancreas is lost when its reaction becomes acid.

It was known to Claude Bernard that the pancreas only possesses its power of decomposing fats so long as it is fresh. Bidder and Schmidt[1] pointed out that the power which the pancreas possesses of decomposing the neutral fats is inhibited by the presence of acids, and this has been particularly insisted upon by Grützner[2]. Glycerin extracts of pancreas are usually inactive as regards fats, and this because the acidification of the gland has occurred before the glycerin could have access to it.

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[1] Bidder and Schmidt, *Die Verdauungssäfte*, p. 250.

[2] Grützner, 'Notizen über einige ungeformte Fermente des Säugethierorganismus,' Pflüger's *Archiv*, Vol. xii. (1876), p. 302 et seq.

Grützner's method of preparing the fat-decomposing ferment.

The perfectly fresh pancreas is crushed with glass powder in a mortar, and mixed with a solution composed of 90 c.c. of glycerin and 10 c.c. of a 1 per cent. solution of Na_2CO_3, in the proportion of 30 c.c. of the glycerin solution to 3 grms. of pancreas, the contact being allowed to continue for not longer than four or five days as, after that time, in spite of the alkali added, the reaction becomes acid.

Grützner's method of observing the comparative fat-decomposing activity of pancreatic extracts.

Given various extracts prepared by the above method, Grützner ascertained their activity as follows:—He made an emulsion by mixing 10 parts of oil of almonds with 5 parts of gum arabic and 35 parts of water, and prepared a neutral solution of litmus, of such strength and reaction that the solution when contained in test-tubes having a diameter of 12 millimetres and placed opposite white paper, had the colour of violets. Several test-tubes had 10 c.c. of the litmus solution added to them, and were then mixed with 5 drops of the above emulsion. Successively increasing quantities of the glycerin extract to be tested (say 2, 4, 6, 8 drops) were then added to different test-tubes, which were at once heated by being plunged into water at 37° C. In three or four minutes the tubes were all examined, and the number of drops which had been added in order to redden the litmus noticed. By having several sets of such experiments, one for each extract to be tested, it is easy to determine the comparative richness in ferment. Grützner's experiments have been repeated by the Author, and have left no doubt on his mind as to the existence of the fat-decomposing ferment.

The pancreas and pancreatic extracts possess the property of decomposing acetic ether

The power of the pancreas and of suitable extracts to decompose the neutral fats suggested the possibility that they might likewise decompose such a body as acetic ether, and experiment has proved the truth of the surmise[1].

Hypothesis of a Ferment which decomposes Fats.

As has been already stated, Bernard explains both the emulsionizing and the acidifying of the neutral fats by the pancreatic juice, or by the pancreatic tissue, as due to a special ferment, the so-called 'emulsive ferment.' For this ferment Dr Sheridan Lea suggests the name 'piolyn.'

No method of separating the ferment, even in a condition of approximate purity, is however known. In future researches in this direction it will be well to bear in mind, in addition to the influence of acids in destroying permanently the fat-decomposing power, and to the fact that by Grützner's method active solutions of ferment of considerable energy can be obtained, that according to Danilewski, pancreatic extracts which possess fat-decomposing powers lose them on agitation with magnesia.

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[1] Heritsch, 'Ueber die zersetzende Einwirkung des pankreatischen Glycerinauszuges auf Essigsäureäther,' *Centralblatt f. d. med. Wissenschaft*, for 1875, No. 28.

Roberts's objections to the hypothesis of a fat-decomposing ferment.

Roberts has expressed himself as doubtful of the existence of a fat-decomposing ferment. His objections are based upon his having been unable in any of his experiments to obtain, either with extracts of pancreas prepared by means of various agents, or with the pancreatic tissue, a decomposition of neutral fats. He has not, in fact, been able to observe the fundamental fact discovered by Claude Bernard. The failure of Roberts is doubtless to be explained by his having, probably in every case, employed pancreatic glands of slaughtered animals and which were not, in the physiological sense, fresh, but had undergone acidification. For in reference to the fundamental fact of the acidification of the neutral fats, there is not the slightest doubt; it is a fact which has been confirmed by the testimony of many independent and reliable witnesses, as of Bidder and Schmidt, Heidenhain, Bernstein, Hüfner, and Grützner, and it is a fact which, if the precautions indicated by Grützner are observed, no one will have any difficulty in confirming for himself.

May not the fat-decomposing ferment be a formed ferment?

Whilst Roberts failed to observe the rapid decomposition of fats by pancreatic tissue or pancreatic extracts, he sometimes succeeded in observing an acidification concurrently with the development of organisms.

The researches of Pasteur and others have taught us that acid fermentations arise under the influence of formed ferments (e.g. the lactic fermentation under the influence of '*bacterium lactis*'), and the question arises whether the fat-decomposing ferment of the pancreas may not be a formed ferment. We answer the question in the negative on the following grounds:

Firstly. Perfectly clear glycerin extracts of pancreas may be obtained which possess the fat-decomposing powers.

Secondly and thirdly. The action is one which is, as Bernard shewed, almost instantaneous, and in this respect resembles actions exerted by other unformed ferments, and is unlike those which are dependent upon organized forms. It takes place, moreover, in presence of such bodies as thymol, which effectually prevent the action of the organised ferments.

Grützner's observations on the richness of the pancreas in fat-decomposing ferment.

Grützner has found that the richness of the pancreas in the fat-ferment varies, and in the same sense as its richness in diastatic and proteolytic enzymes. Thus the pancreas of a dog is poorest in the fat-ferment about six hours after a rich meal. Thereafter the amount increases up to the fortieth hour, so that the pancreas of fasting animals is richest in the fat-ferment.

Grützner's hypothesis as to the seat of formation of the fat-decomposing ferment.

Grützner believes that the central zones of the pancreatic cells not only form the proteolytic enzyme of the pancreas, but that the fat-decomposing and diastatic enzymes are also formed there.

The part which the emulsionizing and fat-decomposing properties of the pancreatic juice plays in intestinal digestion will be treated of in Chapter VI.

SECT. 6. THE PROTEOLYTIC ENZYME—TRYPSIN.

Historical notes on the discovery of the property of the Pancreatic Juice to dissolve and digest Proteids.

The statements of Purkinji and Pappenheim.

It was, we are informed by Corvisart, discovered by Purkinji and Pappenheim in 1836 that the pancreas can furnish extracts which possess the power of dissolving proteids[1], but the discovery seems to have passed altogether unnoticed[2].

Claude Bernard's statements.

Claude Bernard does not appear to have known the researches of the authors just mentioned. In his writings on the pancreas, he, however, stated that the pancreatic juice by itself has no action upon proteids, but that it is able to dissolve them either when they have first of all been subjected to the action of bile or when it acts in conjunction with bile. He pretended, indeed, that by mixing bile and pancreatic juice a liquid is obtained possessed of new properties, being capable not only of emulsionizing and decomposing fats and of converting starch into sugar, but of dissolving proteids[3]. To this function of the pancreatic juice Claude Bernard, however, attributed but little weight; he, indeed, subordinated in a remarkable degree the other functions of the pancreas to the one which he had himself discovered, viz. its action upon fats.

Corvisart's discoveries[4].

The whole merit of clearly pointing out the great error into which Claude Bernard had fallen in denying a proteolytic action to the pancreatic juice *per se*, belongs to Lucien

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[1] The references given by Corvisart are the following: Burdach, *Traité de physiologie*, traduit par Jourdan sur la deuxième édition, IXe vol. p. 317. Paris, 1841. D'après Froriep's *Notizen*, Vol. 14.

[2] This statement is made because of the silence of all the important systematic writers on this matter.

[3] Claude Bernard, *Leçons de Physiologie Expérimentale*, Vol. II. (1856), p. 440 *et seq.*

[4] Corvisart, *Sur une fonction peu connue du Pancréas: La Digestion des Aliments azotés*, Paris, 1857–58.

Corvisart. He pointed out that this juice possesses extraordinary power of digesting proteids at the temperature of the body, and that it possesses this power in neutral, alkaline, and even in acid fluids. Within certain limits he was correct in his assertion, though, as will be shewn in the sequel, pancreatic proteolysis ceases in a too acid medium.

The energy with which pancreatic juice can act was shewn to be indeed striking by Corvisart, who asserted that 15 grammes of fluid obtained during the sixth, seventh and eighth hours of digestion, from a temporary pancreatic fistula, digested completely in two hours five grammes of blood-fibrin, and in four hours as much boiled blood albumin.

Corvisart found that infusions of the fresh pancreas made by digesting the minced gland in twice its volume of water at 40°, for two hours, possessed in an intense degree the proteolytic power, being able to dissolve from 40 to 55 grammes of moist coagulated blood albumin. He shewed that the pancreatic juice and extracts of pancreas not only dissolved proteids but actually converted them into peptones having the characters of the gastric peptones.

Corvisart further found that the precipitate produced by alcohol in an active infusion of pancreas, and which is in main part soluble in water, yields a solution which possesses, approximately, the same power of digesting proteids as the infusion which had yielded it.

The period of digestion at which an animal is killed, Corvisart found, has a great influence upon the activity of the pancreatic extracts, the most active being obtained between the sixth and ninth hours after a full meal.

Corvisart's results contradicted by some observers.

Corvisart's results were, however, neither generally accepted nor generally confirmed by experimenters who attempted to repeat his observations. Thus Keferstein and Hallwachs[1] and O. Funke[2] denied the proteolytic power of the pancreatic juice and of infusions of pancreas, asserting that when proteids are dissolved it is in consequence of a process of putrefaction.

Meissner's observations and errors.

Meissner[3], however, corroborated the main statements of Corvisart as to the powerful proteolytic action of the pancreatic juice and of infusions of the pancreas of animals during digestion, but he fell into the strange error of believing that a slightly acid reaction was absolutely essential to pancreatic, as it is to gastric, proteolysis.

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[1] Keferstein and Hallwachs, "Ueber die Einwirkung des pankreatischen Saftes auf Eiweiss." *Nachrichten von der Kl. Ges. d. Wiss. zu Göttingen*, 1858. No. 14 (quoted at second-hand).

[2] Funke, quoted by Meissner.

[3] Meissner, "Verdauung der Eiweisskörper durch den pankreatischen Saft." *Zeitschrift f. rat. Medizin* von Henle u. Pfeufer, 3rd Ser. Vol. 7 (1859), p. 17.

Danilewsky's researches.

In a research carried on under the direction of Kühne in the Laboratory of the Pathological Institute of Berlin, Danilewsky[1] thoroughly confirmed Corvisart's statements as to the powerfully proteolytic action of infusions of pancreas and devised a method whereby he was enabled to separate approximately the diastatic and proteolytic ferments of the pancreas. He found that the latter, when separated, was only capable of digesting proteids in neutral or feebly alkaline solutions, digestion being inhibited both by the presence of free alkali and of free acid.

Kühne's earlier researches.

In spite of the confirmation which Corvisart's researches had received from several independent observers, as Schiff, Meissner and Danilewsky, the effect of the contradictory statements of others, as Keferstein and Hallwachs, O. Funke, and Skiebitzki had been such that in 1867, when Kühne published his first and most important paper, it could not be said that the proteolytic function of the pancreatic juice was an admitted scientific fact.

In this paper[2] Kühne not only fully confirmed the statements of Corvisart that the pancreatic juice, weight for weight, has a far greater proteolytic activity than the gastric juice, basing his statements upon observations on 11 dogs in which he had established temporary fistulae, but he announced that the tissue of the pancreatic gland might be employed in effecting the digestion of proteids, instead of the juice. He announced the interesting discovery that when blood-fibrin is subjected to pancreatic digestion, it yields not merely peptones, differing but little from those which result from gastric digestion, but also large quantities of leucine and tyrosine, and in those cases in which the proteolytic action had been accompanied (as commonly occurs when it is long continued) by putrefactive changes, fœtid products occur, from which he, later, separated indol.

The Researches of Heidenhain.

The great interest which had been awakened in the proteolytic activity of the pancreas by the researches of Kühne was intensified by the publication in 1875 of a remarkable Memoir by Heidenhain[3]. In this paper the author described, for the first time, those changes in the secreting cells of the pancreas, corresponding to different states of activity, which have already been referred to (p. 197), but further announced the remarkable fact that the fresh pancreas does not contain the proteolytic ferment ready formed, but a body which Heidenhain termed *zymogen*, which may be extracted from the gland, and which under suitable treatment yields the proteolytic ferment. We shall describe this body which is the antecedent of the proteolytic ferment under the name of the 'zymogen of trypsin,' the latter being the name by which Kühne has denominated the proteolytic pancreatic ferment.

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[1] Danilewsky, "Ueber specifisch wirkende Körper des natürlichen und künstlichen pancreatischen Saftes" (aus dem chemischen Laborat. d. patholog. Instituts zu Berlin), *Virchow's Archiv*, Vol. 25 (1862), p. 267.

[2] Kühne, "Ueber die Verdauung der Eiweissstoffe durch den Pankreassaft." *Virchow's Archiv*, Vol. 39 (1867), p. 130. It must not be forgotten that both Danilewsky's and Cohnheim's researches on the enzymes of the pancreas had been carried out in the Chemical Laboratory of the Pathological Institute, then under the direction of Kühne.

[3] Heidenhain, "Beiträge zur Kenntniss des Pancreas." Pflüger's *Archiv*, Vol. x. pp. 557—632.

The Zymogen of Trypsin.

The fresh pancreatic tissue is either free from ready formed proteolytic ferment, or only contains traces. A few hours after removal from the body the pancreas, however, always contains trypsin. The change which takes place is associated with the acidification of the gland, and Heidenhain hastened the conversion by treating the gland substance with acids.

The zymogen of trypsin is soluble in glycerin, which may therefore be used to extract it from the gland tissue; it is likewise soluble in water.

The amount of zymogen varies according to the state of activity of the pancreas and corresponds to the histological changes which occur in the gland cells, being largest in amount when the granular inner zone is abundant, i.e. when the gland has been inactive for some time, and being smallest when the gland cells are small, and their inner zone poorest in granules, i.e. at the end of a digestive period.

The zymogen splits up with the development of free trypsin (*a*) in watery solutions, with a rapidity which increases with the temperature, (*b*) in acid watery solutions more rapidly than in neutral, (*c*) in solutions of neutral and of alkaline salts.

Method of preparing solutions of the zymogen of trypsin.

The pancreas is removed immediately after death, covered with glycerin, and thereafter comminuted and pounded in the glycerin. After some days the glycerin solution is decanted and, if needs be, filtered. In neutral glycerin solution, zymogen remains indefinitely undecomposed. According to Kühne, alcohol causes its decomposition and the appearance of the ferment.

It has been asserted that when oxygen gas is passed through solutions of the zymogen of trypsin for a few minutes the ferment is set free, whilst hydrogen gas passed through a similar solution of zymogen has no effect. Solution of hydrogen peroxide acts in a similar way to gaseous oxygen, as also does agitation with platinum black[1].

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[1] Podolinski, *Beiträge zur Kenntniss des pancreatischen Eiweissferments*. Breslau, 1876 (quoted by Heidenhain; Hermann's Handbuch, Vol. v. 1, p. 189).

Trypsin[1].

The earlier attempts to separate the proteolytic enzyme of the pancreas have been already referred to. It has been stated that at first it was believed that the pancreatic juice contained one ferment, which was by most writers termed '*pancreatin*' or '*pancreatinin*' possessed of several different actions.

Through the labours of Danilewsky and Cohnheim, both working under Kühne's direction, it was, however, clearly made out that the different actions depended upon different enzymes, and attempts were made to separate the diastatic from the proteolytic ferment. The former, it seemed, was neither a proteid nor associated with a proteid body, and the latter also, according to Danilewsky, when in a state of quasi-purity, did not yield the proteid reactions. The subsequent researches of Kühne appear to shew that Danilewsky fell into error, the proteolytic enzyme being a highly complex body which when decomposed yields proteids and their derivatives.

Kühne's method of preparing trypsin.

To infusions or extracts of the pancreas rich in trypsin, alcohol is added in large excess. The precipitate (the '*pancreatin*' of the earlier experimenters) is dissolved in water at 0°C. and precipitated by and digested for some time in absolute alcohol. The precipitate is treated with water, which leaves some albumin undissolved, but which dissolves trypsin and a proteid closely resembling, though not identical with native albumin, and to which Kühne gives the name of '*Leukoid*.'

From the solution leukoid is precipitated by adding acetic acid until it amounts to 1 per cent. Having thus got rid of the greater part of the leukoid, the liquid is rendered slightly alkaline by means of sodium hydrate, and the precipitate which then falls is filtered off. The solution is concentrated at a temperature of 40° C., when the greater part of the tyrosine which is always present, separates, and to the solution, alcohol is added which throws down the enzyme still contaminated with leukoid, peptones, some tyrosine, &c. From these bodies it is freed by solution in water, dialysis, and precipitation by alcohol, the whole series of processes being repeated several times if necessary.

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[1] *Etymology of the word Trypsin.*

In one of his earlier papers on the proteolytic enzyme of the pancreas, Kühne announced that he had named this body (the knowledge of which we certainly owe to him) *trypsin*, without, however, vouchsafing any information as to the etymology of the word.

The only printed information on the matter occurs as a foot-note to a paper by (a pupil of Kühne's) Neumeister, "Zur Physiologie der Eiweissresorption und zur Lehre von den Peptonen" (*Zeitschr. f. Biologie*, Vol. 27 (1890), p. 345. Neumeister says, referring evidently to what is common knowledge in Heidelberg, "'Trypsin' wird bekanntlich von *θρύπτομαι*, zerfalle, abgeleitet, weil dieses Enzym die Eiweisskörper sowohl im mechanischen als auch im chemischen Sinne zum Zerfall bringt." Obviously, however, it must be from the active *θρύπτω*, to break in pieces, that the word is derived. The rendering of the Greek *θ* by the *t* in 'trypsin' is due to the fact that *θ* is always pronounced by Germans as a hard unaspirated *τ*.

Trypsin is very soluble in water, but it is insoluble in alcohol and, strangely, in pure glycerin. The watery solution is not decomposed by long digestion at 40° C., and when evaporated it yields a translucent, non-crystalline, yellowish, solid residue. Trypsin may be long digested with solutions of sodium hydrate at 40° C. without undergoing decomposition.

When boiled, trypsin yields about 20 per cent. of albumin and 80 per cent. of peptone (antipeptone).

In watery solution pure (?) trypsin is able to dissolve large quantities of raw fibrin with surprising rapidity, indeed well-nigh instantaneously.

Method of obtaining an active solution of trypsin from the pancreas of dead animals.

1. *Heidenhain's Method.* A very active glycerin solution of trypsin can be obtained by the following method which is based upon the behaviour of the zymogen of the proteolytic enzyme, to weak acids:—

A dog is killed from 18 to 20 hours after a full meal of meat and the pancreas having been carefully removed is weighed and pounded in a mortar with ground glass; the comminuted mass is allowed to remain at the temperature of the laboratory for 24 hours and is then well mixed with 1 c.c. of dilute acetic acid (1 per cent.) to each gramme of pancreas. To each part by weight of the acid mixture there are added 10 parts by weight of glycerin. In three days the glycerin solution may be filtered from the insoluble residue.

2. *Roberts's Method.* This has been described under the head of the Diastatic Enzyme (p. 204).

3. *Kühne's Methods of preparing active Solutions of Trypsin.* The complexity of the method of preparing a solution of pure (?) trypsin is such that Kühne has devised methods by means of which very active solutions of trypsin may be made at any time from pancreatic tissue prepared according to a method of his own, and which admits of being indefinitely preserved.

Preliminary treatment of pancreatic tissue.

The fresh pancreas of slaughtered animals, that of the ox being generally used, is freed from adhering fat and connective tissue, and is then minced and digested first with cold alcohol, and afterwards repeatedly extracted with boiling ether in one of the many forms of fat extraction-apparatuses. The insoluble residue is then exposed to the air, so as to allow the ether to evaporate, when there is left a white friable solid mass. This may be kept indefinitely, and made use of to prepare solutions of trypsin. It is now an article of commerce in Germany, being sold by those who deal in reagents and materials necessary to the physiological chemist, under the term of Kühne's 'Pankreas-pulver' (or *Pancr. siccum purissimum, nach* Kühne).

Kühne's first solution of trypsin[1].

One part of the dried pancreas, prepared as above described, is digested at 40°C. for 3 or 4 hours with 5—10 parts of a solution of salicylic acid containing 0·1 of the acid per cent. The mixture is then passed through a linen filter and the filtrate, after it has been allowed to cool, is filtered through paper. If, later on, tyrosine crystallises out, the process of filtration is again repeated. If the process be successful, a small quantity of the solution should cause a previously warmed flocculus of fibrin to commence to break down in one minute, and should have reduced it to a thin magma in five minutes.

Kühne's second solution of trypsin.

100 grammes of dried ox pancreas which has been purified by the previously described alcohol- and ether-treatment are digested for twelve hours at 40° C. with 500 grammes of a 0·1°/$_0$ solution of salicylic acid. The mixture is filtered through gauze. The residue is now suspended in 500 c.c. of a 0·25°/$_0$ solution of sodium hydrate, and thymol having been added, is digested for 12 hours longer. The first salicylic solution is likewise digested for 12 hours after it has been neutralised and rendered alkaline to the same extent with $NaOH$.

After filtering and expressing the insoluble matter, both the solutions are united. It was found by Kühne that of 100 grammes of solid pancreas which had been worked with the undissolved residue, after drying, weighed 12 grammes. It contained the nuclein, the collagen, and the undissolved portion of the elastin of the pancreas.

SECT. 7. THE CONDITIONS NECESSARY FOR, AND THE PRIMARY PRODUCTS OF TRYPSIN-PROTEOLYSIS.

The influence of reaction on the digestive activity of trypsin.

It was stated by Corvisart that pancreatic juice could effect the solution of proteids in a medium of which the reaction might be neutral, alkaline or even acid. Meissner afterwards pretended that an acid reaction was necessary. Danilewsky, who had worked under Kühne, stated that fibrin is only dissolved by the pancreatic juice when the solution is neutral or feebly alkaline, a small quantity of alkali hastening the process, a large quantity arresting it.

The more recent researches of Kühne have shewn that an alkaline reaction does, in fact, aid the proteolytic action of trypsin, its activity being greatest, *cæteris paribus*, in solutions containing about 1 per cent. of Na_2CO_3. The pancreatic juice itself is a powerfully alkaline solution, and its alkalinity depends upon the above-mentioned salt. There can be no doubt, however, that trypsin besides acting in an alkaline, can also do so in a neutral, medium and in one whose reaction is feebly acid. The conditions for its activity are therefore seen to be such as enable it to exert its action under the three possible sets of conditions which may and do prevail under varying circumstances, in the small intestine.

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[1] v. Kühne, "Verwendung der Verdauung in der Gewebsanalyse," *Untersuchungen aus dem physiologisch. Institute der Universität Heidelberg*, Bd. 1, 1878, Heft II. S. 222.

Kühne asserted[1] that trypsin is injuriously affected by the presence of hydrochloric acid when it is in greater proportion than 0·5 per 1000, and the statement is correct, inasmuch as some weakening of the digestive action results. It has, however, since been shewn[2] that trypsin can exert its solvent action on fibrin under conditions which would formerly have been considered impossible. C. A. Ewald has observed trypsin proteolysis of fibrin[3] to go on in a liquid containing 0·3 per cent. of HCl, and the observation has been, in a measure, confirmed by Mays, in a research conducted in Kühne's laboratory[4].

It appears, however, that digestion of fibrin by trypsin can only go on in dilute hydrochloric acid containing 0·3 per cent. of the acid, if there be a large quantity of fibrin present. As will be pointed out again, it is unquestionable that trypsin is gradually destroyed by dilute acids, the researches of Langley[5] having fully confirmed the original statements of Kühne, by shewing that a glycerin extract of the pancreas when warmed for two and a half hours with a solution containing 0·05 per cent. of HCl, loses a very appreciable amount of its trypsin.

The influence of temperature on the digestive activity of trypsin.

All observers have agreed in stating that the activity of trypsin increases, within certain limits, with the temperature.

Roberts[6] has carefully studied the influence of temperature upon the activity of trypsin, and states that this increases to 60° C. and then rapidly falls, all action ceasing between 75° C. and 80° C.

The General Phenomena of Proteolysis by Trypsin.

Since the first careful study by Kühne of the action of trypsin on proteids, blood-fibrin—the very proteid which Kühne used in his earlier experiments—has been chiefly employed for the same purpose, and we shall, on this account, in our brief sketch of the proteolytic action of trypsin confine our attention, in the first instance, to fibrin.

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[1] Kühne, *Verhandlungen des Naturhist. Med. Vereins zu Heidelberg*, Bd. 1.

[2] Engesser, "Beiträge zur therapeutischen Verwendung d. Bandspeicheldrüse von Schlachtthieren und deren Präparate," *Deutsch. Archiv f. klin. Medicin*, Bd. 24, S. 539.

[3] C. A. Ewald, "Das Engesser'sche Pankreaspulver." *Zeitschrift f. klin. Med.*, Bd. 1, S. 615.

[4] Karl Mays, "Ueber die Wirkung von Trypsin in Säuren und von Trypsin und Pepsin aufeinander." *Untersuchungen a. d. physiolog. Institut in Heidelberg*, Bd. 3, S. 378.

[5] J. N. Langley, "On the Destruction of Ferments in the Alimentary Canal." *Journ. of Physiology*, Vol. 3, p. 263.

[6] W. Roberts, M.D., F.R.S., "On the Estimation of the Amylolytic and Proteolytic Activity of Pancreatic Extracts." *Proceedings of the Royal Society*.

Blood-fibrin, obtained by stirring recently shed blood with twigs and then washing in a stream of water until it has become perfectly white, is very readily digested by trypsin. The fibrin may be in a raw or thoroughly boiled condition, some observers having employed it in the one, others in the other condition. Thoroughly boiled fibrin whilst it is somewhat less rapidly acted upon than the unboiled, presents the great advantage that it has been freed from organic germs, and especially from putrefactive bacteria, by the process, so that by making use of it, it is easy to conduct a pancreatic digestion so as to avoid the development of putrefaction and the subsequent complications which the putrefactive process introduces when its influence is superadded to that of trypsin.

As will be seen in the sequel, it is, however, easy to inhibit putrefactive changes during the course of a pancreatic digestion, by the use of salicylic acid or of thymol, agents which whilst they prevent the development of putrefactive germs and therefore of putrefaction, exert no unfavourable influence on the pancreatic enzymes which in the presence of these chemical agents, manifest their characteristic activities, unchecked.

When boiled fibrin is placed in a 1 p.c. solution of sodium carbonate, Na_2CO_3, at the temperature of 40° C., it remains unchanged. If a small quantity of an active glycerin extract of pancreas, or some other active preparation of trypsin, be added to the fluid, solution very soon commences.

In this and other similar experiments, that most admirable preparation known as Benger's 'Liquor Pancreaticus' may be used with great advantage. Apart from its great and uniform activity, there is no risk with it (if scrupulous attention to cleanliness have been observed in boiling the fibrin, and solution of Na_2CO_3, sterilising the flask in which the digestion is carried on, &c.) that the digestion will, against the wish of the experimenter, become putrefactive.

It is to be noted that, under the influence of trypsin, the fibrin does not undergo any preliminary process of swelling, as is the case when fibrin is subjected to the action of pepsin and hydrochloric acid.

As the solvent action of trypsin proceeds, the fibrin does not become translucent, but its margins become more and more eroded, and it diminishes in size, gradually disappearing, and often leaving scarcely any residue, although as a rule a certain amount of a greyish, pulverulent residue is obtained when a considerable quantity of fibrin is digested by means of trypsin.

The course of pancreatic digestion proceeds, as has been already said, in a very different manner when putrefactive changes are allowed to set in, and in studying uncomplicated digestion by trypsin it is now usual, according to Kühne's directions, to employ salicylic acid and thymol, agents which, in certain proportions, do not affect the action of the pancreatic enzymes, whilst they absolutely check the development of those organisms which are the essential cause of putrefaction. We are thus able to study the process of the decomposition of the proteids by trypsin, uncomplicated by the presence of products of bacterial action.

The primary products of the action of trypsin.

The first products of the action of trypsin upon the proteids are, as has been already repeatedly stated, the same as those which result from the action of acids and pepsin at a suitable temperature, to wit, hemi-albumose and anti-albumose, and these by the continued action of trypsin are converted into hemi-peptone and anti-peptone.

We have already pointed out that it is a characteristic of hemi-peptone that under the influence of trypsin in alkaline media it undergoes decomposition, yielding, in the first instance, such bodies as leucine, tyrosine and glutamic acid, whilst anti-peptone absolutely resists the action of trypsin and may thus be obtained with comparative ease free from all traces of hemi-peptone.

Quantities of products obtained in the decomposition of products by trypsin.

It is of interest to ascertain so far as possible the quantities of the chief products obtained when a proteid is subjected to digestion with trypsin. One of the earliest of Kühne's experiments furnishes us with this information.

A quantity of blood-fibrin, corresponding to 382 grms. of the dried substance, was digested in 6 litres of water at a temperature of about 40° C., in the presence of Na_2CO_3, through the agency of chopped-up dog's pancreas weighing 55 grms.; the solid matter in the pancreas used was calculated to be 15·2 grms. At the completion of the digestion it was found that 343·7 grms. of fibrin had passed into solution. From the latter the peptone obtained by precipitation with alcohol amounted to 211·2 grms.; further, there were obtained 13·3 grms. of tyrosin and 31·6 grms. of leucin. In this digestion the amount of peptone obtained amounted to 53 per cent.; the leucine to 7·9 and the tyrosine to 3·3 per cent. of the combined water-free fibrin and gland digested, whilst about 20 per cent. of other unknown soluble products were formed.

Normal trypsin digestion not associated with the evolution of gases.

Under the influence of bacterial action, as we shall afterwards have to shew, the digestion of proteids by trypsin is associated with the evolution of large quantities of inflammable and fœtid gases. When however bacteria are excluded, the decomposition of proteids by trypsin proceeds without the evolution of gases, as has been shewn by the researches of Hüfner[1]. This observer found that in the absence of bacteria there is, during prolonged digestion by trypsin, an absorption of oxygen from the surrounding medium (as from the air of the flask in which the digestion is carried on) and a development of CO_2, the amount of this gas evolved being however very small. We shall afterwards draw attention to the results of Nencki's[1] observations on the gases formed during pancreatic digestion associated with putrefaction.

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[1] Hüfner, 'Ueber ungeformte Fermente und ihre Wirkungen bei der Pankreasverdauung.' *Journal für prakt. Chemie*, Vol. x. p. 1.

We have now to study in succession the chief products of the action of trypsin on proteids.

SECT. 8. ANTI-PEPTONE RESULTING FROM THE ACTION OF TRYPSIN (SYN. TRYPTONE).

Our knowledge of the mode of preparation, composition and reactions of anti-peptone, the product of the prolonged action of trypsin acting in an alkaline medium, at a suitable temperature, is based entirely on the researches of Kühne, and of Kühne and Chittenden. The most recent information concerning anti-peptone is contained in a paper to which reference has already frequently been made in the preceding pages[2].

Mode of preparation of anti-peptone from blood-fibrin.

Blood-fibrin is the most convenient proteid to employ in the preparation of anti-peptone, or indeed in general whenever the chief products of decomposition of the proteids by trypsin are to be studied.

In his experiments Kühne took well-washed blood-fibrin, boiled it in water, then in alcohol, and extracted it with ether. The fibrin which had thus been purified was again boiled in water and then subjected to the digestive process.

In his earlier researches on pancreatic digestion, Kühne prevented the onset of putrefaction by employing an extract of pancreatic gland made by digesting the pancreas in a solution of salicylic acid. Thus in one experiment he digested 800 grms. of pancreas at 40° C. in 2 litres of water containing 4 grms. of salicylic acid, and employed the solution of ferment thus obtained to effect the digestion of large quantities of fibrin.

In his more recent experiments, in association with Chittenden, Kühne has carried on his digestions with the aid of extracts of pancreas which, *mutatis mutandis*, were made essentially in the manner described at page 222; viz. by first digesting pancreas which had been dehydrated and freed from fat by treatment with alcohol and ether, in 0·1 per cent. solution of salicylic acid, and afterwards digesting the undissolved residue of the gland in 0·25 per cent. solution of sodium hydrate to which thymol had been added.

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[1] Nencki, 'Ueber die Zersetzung der Gelatine und des Eiweisses bei der Faülniss mit Pancreas.' Maly's *Jahresbericht*, Vol. VI. (1876), p. 31.

[2] Kühne and Chittenden, 'Ueber die Peptone.' *Zeitschrift für Biologie*, Vol. XXII. (1886), see p. 434 *et seq*.

The following actual examples taken from Kühne and Chittenden's researches will explain the processes to be followed in preparing anti-peptone.

300 grms. of dry fibrin, which had been purified as stated above and as a last process had been boiled in water and the water expressed with the hand, were found to weigh 970 grms. This quantity of moist fibrin was placed in three litres of a 0·25 per cent. solution of sodium hydrate, to which ½ per cent. of thymol had been added. The infusion obtained (as explained previously, viz. by the salicylic acid and NaHO and thymol method) from 88 grms. of pancreas was then added to the fibrin and the whole mixture digested at 40°C. during six days. The very first day nearly the whole of the fibrin was dissolved, though a little yet remained undissolved and floated on the surface of the liquid.

The digested liquid was slightly acidulated with acetic acid, boiled, and filtered through cloth, and the filtrate concentrated to 1 litre. On cooling, there crystallised out the greater part of the leucine and tyrosine which had been formed, and to the brownish syrup which was drained off alcohol was added until a precipitation of peptones commenced. The liquid was then boiled, to dissolve any precipitated peptone, and set aside again to crystallise. The filtrate from the second crystallisation, now tolerably free from amido-acids, was saturated with ammonium sulphate. This reagent besides precipitating any albumoses which may be present, carries down, according to Kühne and Chittenden, many accidental impurities, and seems to precipitate very perfectly the trypsin which may yet be present in the liquid. The filtrate from the ammonium sulphate precipitate was then freed from a great part of the salt by concentrating it, cooling and allowing the salt to crystallise out, the separation being aided by the addition of considerable quantities of alcohol. In order finally to free the peptone from the ammoniacal salt, the same process was followed as for the preparation of amphopeptones from fibrin (see page 137); i.e. the solution was boiled with barium hydrate and barium carbonate, and after expulsion of the whole of the ammonia by boiling, the compound of anti-peptone and barium was decomposed by means of sulphuric acid. The peptone was repeatedly precipitated with alcohol. When dried at 105° the anti-peptone obtained from 300 grms. of fibrin weighed 120 grammes.

Kühne and Chittenden found as much difficulty in drying anti-peptone as they had encountered in the case of ampho-peptones.

Reactions of solutions of anti-peptone free from albumoses.

Anti-peptone, like ampho-peptone, is only completely precipitated from its solutions by the following reagents:—tannin, a solution of iodide of mercury in potassium iodide; and almost completely precipitated by phospho-tungstic acid, phospho-molybdic acid and picric acid.

Kühne and Chittenden have compared the action of many reagents on anti-peptone and ampho-peptone, and the results of these observations are exhibited below[1].

Reactions of Peptones free from albumoses and purified by phospho-tungstic acid.

In 5 per cent. solution, after being made noticeably alkaline with a trace of sodium carbonate.

	Fibrin anti-peptone.	Fibrin ampho-peptone.
Acetic acid and potassium ferrocyanide.	At first perfectly clear, later trace of opalescence.	The same.
Neutral lead acetate.	First drop, 0; more, turbidity.	The same, but much weaker.
Basic lead acetate.	Turbidity immediately; more, strong turbidity.	The same, but weaker.
Mercuric chloride.	First drop, 0; more, strong turbidity.	Turbidity immediately, growing stronger.
5 per cent. cupric sulphate.	At first clear; more, slight turbidity disappearing with great excess.	Nothing.
5 per cent. platinum chloride.	Only excess, strong turbidity.	Nothing.
Chromic acid.	Nothing.	Nothing.
Ferric chloride.	A trace gives turbidity vanishing with the least excess.	Nothing.
Glacial acetic acid and conc. sulphuric acid.	Brownish red.	The same.
Nitric acid.	The colour changing yellow in the cold.	The same.
Boiling with conc. hydrochloric acid.	The colour becomes slightly darker.	The same.
Millon's reaction.	At first a heavy white precipitate; on warming, dirty yellow or reddish.	The same, then beautiful red colour.

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[1] Kühne and Chittenden, 'Peptones.' *Studies from the Laboratory of Physical Chemistry of Yale University* (for the year 1885—86), see p. 40. This paper is a

Employment of trichloracetic acid as a precipitant of albumoses and peptones.

Trichloracetic acid, CCl_3COOH, has of late been employed as a precipitant of albuminous bodies generally, both in qualitative and quantitative analyses[1]. Thus it has been used in the quantitative analysis of albumin in urine and of casein in milk.

It would appear that this acid throws down both albumoses and peptones, the latter less perfectly than the former. In dilute solutions of peptones, trichloracetic acid fails to produce a precipitate.

SECT. 9. ENUMERATION OF THE PRODUCTS (OTHER THAN ALBUMOSES AND PEPTONES) OF THE ACTION OF TRYPSIN UPON THE ALBUMINOUS BODIES.

General Observations.

Besides the bodies which have been already described as albumoses and peptones, trypsin when it acts upon the albuminous bodies, even in the absence of putrefactive bacteria, gives rise to the appearance of numerous substances, the products of a far-reaching decomposition of the complex albuminous molecule, and which are formed altogether independently of oxidation:—products which are likewise obtained, some in one case, some in another, when the same bodies are acted upon by various reagents which are capable of effecting their hydrolytic decomposition. Thus under the action of solutions of barium hydrate at high temperatures, by fusion with caustic alkalies, by boiling with dilute sulphuric acid, by boiling with stannous chloride and hydrochloric acid &c., we obtain from the albuminous bodies proper and from the substances closely related to them (which we have designated albuminoid) several definite crystalline products, which vary somewhat with the nature of the decomposing agent, but of which the chief are represented in the products of a normal 'tryptic' digestion carried on *in vitro*, under conditions which exclude the modifying action of putrefactive organisms.

Amongst the usual, we may say constant, products of the action of trypsin on the proteids is a body which when present in a digestive solution causes it to assume a red colour on the addition of chlorine water, and a more or less violet colour when bromine water is added to it. This product will be discussed in the sequel under the name of 'tryptophan' (suggested by Neumeister): a name indicating that it is a colouring matter produced when the proteid molecule is broken down.

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translation of the German original, 'Ueber die Peptone.' *Zeitschrift für Biologie*, Vol. XXII. (1886), see p. 450.

[1] Obermayer, 'Ueber die Anwendung der Trichloressigsäure in der physiologisch-chemischen Analyse.' See a long epitome by Professor Andreasch in Maly's *Jahresbericht*, Vol. XIX. (for 1888), p. 7—10.

The most important of the products, to which reference is now made, and of which a more or less detailed description will follow, are: 1st, amido-acids of the fatty group, to wit:—amido-caproic acid or leucine: amido-valerianic acid: asparagine and glutamic acid. 2nd, an amido-acid of the aromatic series, viz. tyrosine. 3rd, two bases, lysine and lysatinine. 4th, ammonia.

Products (other than Peptones) of the Action of Trypsin on Albuminous and Albuminoid Bodies.

Bodies derived from the Fatty acids.	Bases.	Organic Body of unknown constitution.	Aromatic Bodies.
Amido-caproic acid (Leucine) Amido-valerianic acid (Butalanine) Amido-succinic acid (Aspartic acid) Amido-pyrotartaric acid (Glutamic acid) *Diamido-acetic acid?	Lysine Lysatinine NH_3	Tryptophan	Paroxyphenylamido-propionic acid (Tyrosine) The aromatic bodies which take their origin in the products of pancreatic digestion under the influence of putrefactive bacteria are not enumerated here.

* This acid was found by Drechsel among the products of the decomposition of proteids by stannous chloride and hydrochloric acid. It will, doubtless, be found to be also a product of the action of trypsin.

SECT. 10. THE AMIDO-ACIDS RESULTING FROM THE ACTION OF TRYPSIN ON THE ALBUMINOUS BODIES.

Introductory observations on amido-acids.

If we replace an atom of H in the alcohol-radical of certain acids by the monatomic NH_2-, or amidogen-, group, amido-acids are formed. Thus by replacing in the case of acetic acid, CH_3COOH, one H in the radical CH_3 by NH_2, we obtain $CH_2(NH_2)COOH$, amido-acetic acid, commonly known as glycocoll or glycocine, a body which is one of products obtained from gelatin by long boiling with dilute sulphuric acid, and which also results from the hydrolytic decomposition of hippuric and glyco-cholic acids.

Another example of an amido-acid derived from a fatty acid is afforded by the body leucine, to be immediately considered in detail, and which is an amido-caproic acid.

In the case of each individual acid, the method by which it can be obtained, as well as its special characters, and more important compounds and reactions, will be described with sufficient fulness.

Methods of general application for the separation of the amido-acids.

We shall have to point out that the differences in the solubility of the amido-acids in water and absolute alcohol, respectively, afford a ready means of separating certain of the amido-acids (leucine and tyrosine) which are principal products of pancreatic proteolysis. In addition to these there are, however, others, some of which have already been separated, and others await investigation. The compounds with Cu aid us in this search. All the amido-acids form very sparingly soluble and definite crystalline compounds with copper, which are readily obtained by boiling the aqueous solution of the acid or acids with properly precipitated cupric hydrate $Cu(OH)_2$, filtering and allowing the solution to cool; unless the solution have been very dilute the Cu compound will then separate. These compounds may be purified, the amount of Cu which they contain determined, and, besides, they may be decomposed by H_2S, and the amido-acid obtained in a pure condition[1].

Two important general reactions of amido-acids,

1stly. When treated with nitrous acid the whole of their N is evolved in the gaseous form and an oxy-acid is formed, thus:—

$$(1)\quad \underbrace{CH_2(NH_2)COOH}_{\text{Glycocine.}} + HNO_2 = \underbrace{CH_2(OH)COOH}_{\text{Oxyacetic or glycollic acid.}} + N_2 + H_2O.$$

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[1] Refer to the individual amido-acids for details as to the composition, preparation, purification and decomposition of the Cu compounds.

$$(2)\quad \underbrace{CH_3(GH_2)_3CH(NH_2)COOH}_{\text{a-Amidonormal caproic acid.}} + HNO_2$$

$$= \underbrace{CH_3(CH_2)_3CH(OH)COOH}_{\text{Oxycaproic or leucic acid.}} + N_2 + H_2O.$$

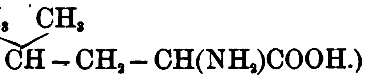
2ndly. When heated with barium hydrate, the amido-acids split up into alcohol bases and carbon dioxide. Thus:

$$\underbrace{CH_2(NH_2)COOH}_{\text{Glycocine.}} = \underbrace{CH_3NH_2}_{\text{Methylamine.}} + CO_2.$$

To conclude these few very elementary observations on the amido-acids, it may be remarked that these bodies in virtue, firstly, of the acid carboxyl-group, and secondly of the basic amido-group which they contain, are at once acids and bases, combining both with bases and acids to form salt-like compounds.

Leucine ($C_6H_{13}NO_2$)[1].

(*a-amido-isobutylacetic acid*



$$\overset{CH_3\quad CH_3}{\searrow\swarrow}\atop{CH - CH_2 - CH(NH_2)COOH}$$

.)

Occurrence in the organism. Leucine has been found in several of the tissues and organs, especially of the glands, of the healthy body: in the spleen, the thymus, the thyroid, in the parotid and submaxillary glands, and above all in the pancreas. It is to the eminent pathologist Virchow[2] that the merit belongs of having first discovered leucine and tyrosine as non-pathological constituents of the pancreas, both in man and the lower animals. He pointed out, further, that the proximate principle which Scherer had separated from the spleen and called *lienin* was undoubtedly leucine. Virchow's short and apparently forgotten paper is of great interest in connection with the history of discoveries in pancreatic digestion. Scherer[3] separated from 20 pounds of the pancreas of the ox, 180 grammes of pure leucine, an amount which corresponds to 1·77 per cent. of the fresh, and to 7·37 per cent. of the dried and water-free glandular tissue. At the time when Scherer analysed the pancreas, the discovery afterwards due to Kühne had not been made, viz. that trypsin, as a result of its digestive activity, splits up the albuminous bodies, and that amongst the most obvious and best characterised products are leucine and tyrosine: further that, as the result of a process of *auto-digestion*, the gland soon after death is found to contain such considerable quantities of these amido-acids as were certainly not existent at the time of death. The question then arises whether any of the leucine found in the pancreas is to be looked upon as a normal constituent *ante mortem*. Numerous facts enable us to answer this question in the affirmative.

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[1] Leucine (from λευκός, bright, clear, white) owes its name to its discoverer, Braconnot, who probably wished to emphasize the origin of the colourless crystals which he had obtained from the black charred products resulting from the action of boiling sulphuric acid on muscle.

[2] Rud. Virchow, 'Zur Chemie des Pancreas.' *Virchow's Archiv*, Vol. VII. (1854), p. 580.

[3] Scherer, Liebig's *Annalen*, Vol. CXII., p. 257.

Radziejewsky, in 1865, under Kühne's guidance[1], made a research with the express object of determining whether leucine and tyrosine are normal constituents of the body; with this object the various organs were removed immediately after animals had been killed, plunged into spirit, then immediately cut into fragments, and pounded with the help of sand. The alcoholic solution was then investigated. It was found that whilst tyrosine occurs normally in no single organ, leucine is a constant constituent, though in different and fluctuating proportions, of the pancreas, the spleen, the lymphatic glands, the salivary glands, the thyroid, thymus and liver[2]. Leucine was neither found in the muscles, lungs and brain, nor in blood, saliva, bile, and urine. The presence of leucine in the kidney and testicle was considered doubtful. Treskin[3], in a research made under Hoppe-Seyler's direction, subsequently discovered leucine in the testicle, and Cloetta[4] discovered it to be a normal constituent of lung tissue.

Leucine, according to v. Gorup-Besanez, has been found in the alimentary canal of the pupae of *Sphinx pinastri* and *Cossus ligniperda*: in arthropoda: in *Astacus fluviatilis*; in spiders, caterpillars and butterflies, and indeed is a frequent constituent of invertebrata[5]. It was found by Professor Zöller of Vienna (together with tyrosine) in the contents of two fossil eggs discovered in the guano of the Island of Chincha (Peru)[6].

Leucine has been found by Gorup-Besanez in seedling vetch plants[7], together with asparagine: by Schulze and Barbieri[8], together with asparagine and tyrosine, in seedling pumpkins.

In pathological conditions, leucine has been found together with tyrosine, in large quantities, in the blood, liver and urine of cases of acute yellow atrophy[9]: in the liver and urine in cases of phosphorus poisoning[1]: in the liver and urine of cases of cirrhosis: more abundantly than in the normal condition, in the liver of cases of typhus and variola. It has been found in the blood of leukaemic patients: in the alvine dejecta of cholera: in pus: in the sputum of cases of pulmonary gangrene; in certain dropsical transudations: in atheromatous deposits.

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[1] S. Radziejewsky, 'Das Vorkommen von Leucin und Tyrosin in normalen Körper' (aus dem chemischen Laboratorium des pathologischen Instituts zu Berlin). *Virchow's Archiv*, Vol. XXXVI. (1866), p. 1—14.

[2] The reader may consult a paper by Rud. Virchow, 'Ueber der Leucin und Tyrosin Abscheidungen an der Leber.' *Virchow's Archiv*, Vol. VIII. (1855), p. 355.

[3] Treskin, 'Bestandtheile der Testikel.' *Pflüger's Archiv*, Vol. V. (1872), p. 122—130.

[4] Cloetta, *Annalen d. Chem. u. Pharm.* Vol. XCIX., p. 289.

[5] v. Gorup-Besanez, *Lehrbuch d. phys. Chem.*, 4th ed. (1878), p. 225.

[6] Zöller, 'Ueber die Zusammensetzung fossiler Eier und verschiedener im Guano gefundener Concretionen.' *Anzeiger der k. Akad. in Wien*, 1874, no. 19. The author has not seen this paper, of which a lengthy abstract appeared in Maly's *Jahresbericht*, Vol. IV., p. 334—337.

[7] Gorup-Besanez, *Ber. d. deutsch. chem. Gesellschaft*, Vol. VII. (1874), p. 146 and 569.

[8] E. Schulze und J. Barbieri, 'Leucin aus Kürbiskeimlingen.' *Ber. d. d. chem. Gesell.* 11, 1238.

[9] Frerich's *Deutsche Klinik*, 1855, no. 31, 'Klinik der Leberkrankheiten,' 1858.

Modes of preparation of leucine and its separation from tyrosine.

1. *By the action of trypsin on proteids.* A large quantity of well-boiled fibrin is digested with a salicylic and thymolised solution of Kühne's dried pancreas (see p. 222), exactly as described for the preparation of antipeptone. The liquid product of digestion is filtered, so as to free it from undissolved fibrin and from accidental impurities; it is feebly acidulated, by means of dilute acetic acid, and boiled. Having filtered off the precipitate which forms, the filtrate is concentrated until it is nearly of syrupy consistence and set aside to cool. After 24 hours a considerable quantity of leucine and tyrosine will have crystallised out. The mother liquor is separated from the crystals and, if necessary, further concentrated. The brown syrupy liquid is then treated with absolute alcohol until a precipitate of antipeptone commences to fall. The alcoholic solution is then heated, so as to dissolve the precipitated peptone, and set aside to cool and crystallise. The mother liquor is again poured off and the crystalline crusts which have separated may be advantageously washed with saturated solution of ammonium sulphate[2]. The mixed leucine and tyrosine resulting from these operations must then be separated and purified.

The simplest method adopted for the separation of leucine from tyrosine rests upon the fact that leucine is very much more soluble both in water and spirits of wine than tyrosine. By boiling the yellow crystalline masses or crusts, composed of impure leucine and tyrosine, with alcohol, the former is dissolved and the latter, in great part, left. From its alcoholic solution, after suitable concentration, leucine will crystallise out, and may be purified by repeated crystallisation from alcohol. From the insoluble residue, tyrosine is obtained by dissolving in a weak solution of ammonia. The solution is allowed to evaporate at ordinary temperature, when tyrosine separates in the crystalline form.

The method of purification of leucine originally devised by Hlasiwetz and Habermann, and to be described in the sequel (p. 236), may be employed for the separation of leucine from the other products of digestion by trypsin.

2. *From glandular organs.* The organ is reduced to a pulp in a mincing or sausage machine, and, if necessary, is mixed with powdered glass and further rubbed down in a mortar. It is digested

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[1] Sotnitschewsky, 'Ueber Phosphorvergiftung' (aus dem physiologisch-chemischen Institute in Strassburg). *Zeitschrift für physiologische Chemie*, Vol. III. (1879), p. 390.

[2] Kühne und Chittenden, 'Ueber die Peptone,' *Zeitschrift f. Biologie*, Vol. XXII. (1886), p. 436.

with cold water with frequent stirring, and the solution is separated from insoluble matter by filtering through calico. The insoluble matters are again treated with cold water. The collected turbid solutions, if distinctly acid, are at once boiled: if not, they are first feebly acidified, by means of acetic acid, and then boiled. After separation of any proteid which has been precipitated, solution of acetate of lead is added and the liquid again filtered. A stream of sulphuretted hydrogen is passed through the filtrate, so as to precipitate the excess of lead. After again filtering, to get rid of the lead sulphide, the filtrate is concentrated to a syrupy consistence and then set aside to crystallise. Any leucine which separates may be then purified by the processes referred to in page 236.

3. *By the action of boiling sulphuric acid and other agents.* It has already been stated that, by the action of various reagents upon the albuminous and albuminoid bodies, the complex molecule is split up and a large number of products are obtained, some of which may be considered as due to the primary decomposition, others as the result of a secondary action of the decomposing agent upon the bodies first formed. Amongst the principal methods which have been employed with success to effect the decomposition of the proteid molecule, and of which the products have been carefully studied, are the following:

1. Heating the substance under investigation with solution of barium hydrate under pressure, at temperatures which have in different researches varied between 100° C. and 200° C. This method has been largely employed by Schützenberger and by Gautier, by Schultze in association with Barbieri and Bosshard, and has led to results which have afforded a considerable amount of information as to the probable structure of the proteid molecule.

2. Boiling the substance for many hours with stannous chloride and hydrochloric acid, an inverted condenser being employed and arrangements sometimes made for excluding atmospheric oxygen. (Hlasiwetz and Habermann[1], E. Drechsel[2].)

3. Heating the substance with bromine water in sealed bottles. (Hlasiwetz and Habermann[3].)

4. By the action of dilute hydriodic acid on coagulated serum albumin. (E. Drechsel[4].)

5. Boiling or fusing the albuminous substances with caustic alkalies.

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[1] Hlasiwetz and J. Habermann, 'Ueber die Proteinstoffe,' *Ann. d. Chem. u. Pharm.*, Vol. CLXIX. p. 150.

[2] E. Drechsel, 'Zur Kenntniss der Spaltungsproducte des Caseins.' Du Bois Reymond's *Archiv*, 1891, p. 254. See also the various papers by Drechsel and his pupils, under the heading Lysine and Lysatinine.

[3] Hlasiwetz and Habermann, 'Ueber die Proteinstoffe,' *Annalen der Chemie*, Vol. CLIX. p. 304 *et seq.*

[4] E. Drechsel, 'Ueber die Einwirkung von verdünnten Säuren auf Albumin. *Ludwig's Festgabe*, p. 83.

6. Long-continued boiling with dilute sulphuric acid; various observers employed acid of different concentration (though the proportion of 1 of acid to 3 of water has been most common), the duration of boiling usually varying between 24 and 36 hours.

By any of these methods of decomposition leucine may be obtained from any of the albuminous or albuminoid bodies, mixed with a large number of other bodies, which are to be looked upon as either primary or secondary products of the decomposition of the proteid molecule. Whilst the first four methods have been employed with the greatest success for the purposes of investigation, the fourth and especially the fifth have been had recourse to when the object has been merely the preparation of leucine and tyrosine, for these two bodies are, with few exceptions, obtained together, and have to be separated one from the other.

As a raw material for preparing leucine the following, amongst other substances, have been chiefly employed: meat, cheese, fibrin, horn, wool, feathers, yellow elastic tissue. The following are the details of the method for obtaining both leucine and tyrosine from horn-shavings:—

1000 grammes of horn-shavings are boiled for 24 hours with a mixture containing 2500 c.c. of concentrated sulphuric acid and 6·5 litres of water, the evaporated water being replaced from time to time.

Thin milk of lime, of uniform consistency, is then gradually added to the acid liquid until an alkaline reaction is obtained, the liquid being continually stirred. The mixture, which necessarily contains a large quantity of precipitated calcium sulphate, is filtered through a jelly-bag; the residue, after squeezing, is boiled with water and again filtered. The mixed filtrates are slightly acidulated with oxalic acid, which precipitates the calcium originally present in solution as calcium oxalate. The liquid being filtered, the filtrate is concentrated until a crystalline pellicle appears, when it is set aside to cool and to crystallise.

The united masses of crystals are dissolved in boiling water; some solution of ammonia is added, and then, with continual stirring, a solution of lead acetate, until the precipitate which forms is no longer brownish but white. The liquid is then filtered and the hot filtrate is acidulated with dilute sulphuric acid, the precipitate of lead sulphate which forms is separated by filtration and the filtrate allowed to cool, when tyrosine separates almost completely and in a pure condition.

The mother liquor from which the tyrosine has been filtered is then treated with sulphuretted hydrogen so as to get rid of the lead which it contains, and, having been again filtered and the excess of H_2S expelled by heat, is concentrated and boiled for a couple of minutes with freshly precipitated cupric hydrate. A dark blue solution is obtained by this process; this is filtered and concentrated, when it deposits sky-blue warty masses of crystals as well as a precipitate having the composition $Cu(C_6H_{10}NH_2O_2)_2$. Both the precipitate and the crystalline masses are decomposed by means of sulphuretted hydrogen, the filtrate from the sulphide of copper formed is, if need be, decolourised by means of animal charcoal, again filtered and sufficiently concentrated, when, on being set aside, the leucine crystallises out. By dissolving the crystals in spirits of wine and recrystallising the substance is still further purified[1].

According to Zollikofer[2] the *ligamentum nuchae* of the ox most readily furnishes leucine. One part by weight of the tissue is boiled for three hours with a mixture of 2 parts of sulphuric acid and 3 parts of water. No increased yield of leucine is obtained if the boiling be further prolonged. The product may be treated exactly as described in the first process.

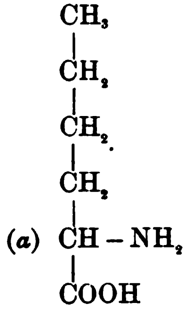
Quantities of leucine obtained from various albuminous and albuminoid bodies.

The yields of leucine when various bodies were treated by the sulphuric acid method were as follows (Erlenmeyer and Schöffer[3]):—Lig. nuchae 35—45 $^0/_0$ of its weight; blood-fibrin 14 $^0/_0$: muscle 18 $^0/_0$: albumin 10 $^0/_0$: horn 10 $^0/_0$. Nencki[4] by the same process obtained from gelatin 1·5—2 $^0/_0$.

Constitution and Synthesis of Leucine.

Leucine is an amido-caproic acid. There are theoretically eight possible caproic acids ($C_6H_{12}O_2$), of which seven have already been prepared, and corresponding to these eight acids, thirty-one possible isomeric amido-caproic acids or leucines. For reasons which space will not permit us to discuss, the leucine which is obtained by the decomposition of the albuminous or albuminoid bodies can only be a derivative of one or other, or of both, of two of the isomeric caproic acids: to wit normal caproic acid or isobutylacetic acid. Further, we are acquainted with facts which prove that in either case the NH_2 group must occupy the *a*-position. Briefly, the constitution of, what we may term, the physiological normal leucine must be represented by one or other of the two appended formulae.

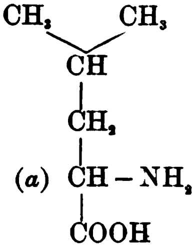
I.



$$CH_3-CH_2-CH_2-CH_2-\overset{(a)}{CH}(NH_2)-COOH$$

a-Amido-normal caproic acid.

II.



a-Amido-isobutylacetic acid.

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[1] The description of this process is taken *verbatim* from Drechsel's 'Anleitung zur Darstellung physiologisch-chemischer Präparate, &c.' The method of separating and purifying leucine from tyrosine given is that employed by Hlasiwetz and Habermann ('Ueber die Proteinstoffe,' *Ann. d. Chem. und Pharm.*, Vol. CLXIX. p. 150).

[2] Zollikofer, 'Beiträge zur Kenntniss d. elastisch. Gewebes.' *Annal. d. Chem. u. Pharm.*, Vol. LXXXII. (1852), p. 162—180.

[3] Erlenmeyer and Schöffer, quoted by Maly, *op. cit.* p. 209.

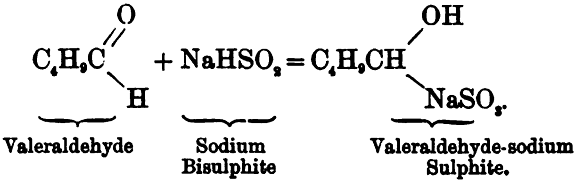
[4] Nencki, *Journ. f. prakt. Chemie*, N. F. Vol. XV. pp. 390—398.

It was until lately surmised, mainly in consequence of the researches of Hüfner[1], that the first of the two formulae represented the constitution of the leucine of the animal body.

Schulze[2] having proved that the leucine which is obtained by the decomposition of vegetable proteids has the constitution of amido-isobutylacetic acid, Hüfner has lately caused a most elaborate investigation to be made in his laboratory by Dr Bernhard Gmelin[3], who has conclusively shewn that leucine of animal origin possesses the same constitution as that derived from vegetable proteids, and that the variations which leucine presents in physical characters—as, for example, in solubility and in its power of rotating the plane of polarization—according to the conditions under which it is formed, are explicable on the theory of physical isomerism, the chemical constitution being the same.

Synthesis of leucine by the action of HCl and HCN on valeraldehyde ammonia (Limpricht).

In a large flask, furnished with an inverted condenser, 125 grms. of potassium bichromate are heated with a mixture of 163 grms. of concentrated sulphuric acid and 1250 c.c. of water to a temperature of 90°. Through a stoppered funnel 100 grms. of amyl alcohol are then allowed to flow in gradually. In this reaction valeric aldehyde ($C_4H_9 . COH$) is formed. This body is now separated by distillation; the distillate is first of all shaken with a dilute solution of sodium hydrate, which is then got rid of by means of a separating funnel, and the impure valeraldehyde is shaken with a concentrated solution of acid sodium sulphite (sodium bisulphite, $NaHSO_3$). Crystals of valeraldehyde-sodium sulphite separate:



$$\underbrace{C_4H_9C\begin{matrix}\diagup\!\!\diagup O\\ \diagdown H\end{matrix}}_{\text{Valeraldehyde}} + \underbrace{NaHSO_3}_{\substack{\text{Sodium}\\ \text{Bisulphite}}} = \underbrace{C_4H_9CH\begin{matrix}\diagup OH\\ \diagdown NaSO_3\end{matrix}}_{\substack{\text{Valeraldehyde-sodium}\\ \text{Sulphite.}}}$$

The crystals are filtered, pressed between filtering paper, and distilled with solution of sodium hydrate, when valeraldehyde is set free and distils over. The distillate is treated with concentrated ammonia and thoroughly shaken, when valeraldehyde-ammonia separates out in the crystalline form.

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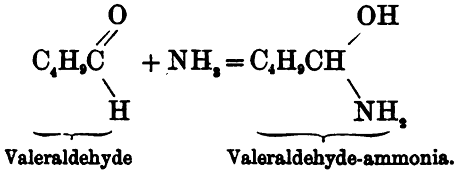
[1] Hüfner, *Journal für pr. Chemie* (2), 1, 6; *Zeitschrift f. Chem.*, Ser. 2, Vol. IV. (1868), p. 391 and 616.

[2] Schulze u. Likiernik, 'Ueber die Constitution des Leucins.' *Ber. d. d. ch. Gesellsch.*, Bd. XXIV. (1891), 4, 669.

[3] Bernhard Gmelin, 'Beiträge zur Kenntniss des Leucins' (Inaugural-Dissertation, Tübingen, 1892).



$$\underbrace{C_4H_9C\overset{\displaystyle O}{\diagup\!\!\diagup}\!\!\underset{\displaystyle H}{\diagdown}}_{\text{Valeraldehyde}} + NH_3 = \underbrace{C_4H_9CH\overset{\displaystyle OH}{\diagup}\!\!\underset{\displaystyle NH_2}{\diagdown}}_{\text{Valeraldehyde-ammonia.}}$$

This compound is now collected on a filter, washed with water and then boiled with a mixture of strong HCN and dilute HCl, in a flask provided with an inverted condenser, until the crystals are completely dissolved. (Two parts of valeraldehyde-ammonia are boiled with 1 part of HCN and an excess of dilute HCl.) It appears that in the first instance a body having the formula $C_{18}H_{33}N_5$ is formed. This body however splits up according to the following equation:—

$$C_{18}H_{33}N_5 + 6H_2O = 3\,(\underbrace{C_6H_{13}NO_2}_{\text{Leucine}}) + 2NH_3.$$

The contents of the flask are now concentrated on the water bath in a draught chamber, due precautions being taken now (as well as in the previous operation) against the possible inhalation of the poisonous vapour of hydrocyanic acid which is evolved abundantly. When the liquid has cooled, ammonia is added, when part of the leucine separates out and is collected on a filter. The mother liquor is evaporated to dryness, the residue extracted with dilute hydrochloric acid, and the solution having been concentrated on the water bath is again treated with ammonia[1].

Physical and Chemical Properties of Leucine.

Crystalline form and melting point.

Pure leucine presents a snow-white appearance and occurs in the form of colourless and light crystalline doubly refracting plates, possessed of a greasy feel and which float on water without being wetted.

When heated to 170° C., leucine melts and then gives off fumes which condense in the cooler part of the tube forming an exceedingly light sublimate, which has been compared to the so-called 'Philosopher's wool,' i.e. sublimed zinc oxide.

When the sublimed leucine is examined microscopically it presents the appearance of thin plates grouped into rosette-shaped masses; the plates when seen edgeways appear to the observer as needles.

When leucine separates from solutions which contain other substances it usually forms crusts which under the microscope are seen to be composed of balls and nodular masses. These balls of leucine are fairly transparent and sometimes present a radiated structure.

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[1] Parkinson, *Ann. Ch. u. Pharm.*, Vol. xc. p. 144. Limpricht, *Ibid.* Vol. xciv. p. 243.

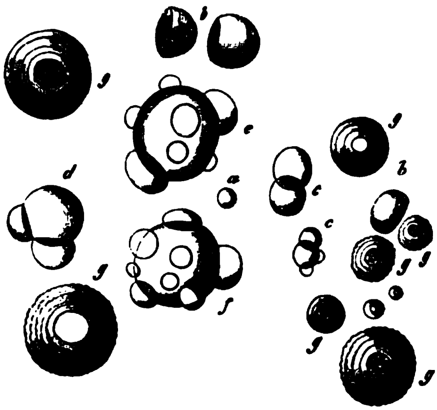

FIG. 15. LEUCINE IN THE FORM WHICH IT USUALLY ASSUMES WHEN SEPARATED FROM THE PRODUCTS OF DIGESTION BY TRYPSIN.

Density. Although crystals of leucine float on water the body is really of higher specific gravity than water. According to Engel and Vilmain, the specific gravity of leucine at 18° C. is 1·293.

Solubility. Leucine, as has already been stated, presents considerable variations in certain of its physical properties according to its origin and perhaps to the mode of preparation employed. These differences are explicable on the hypothesis of the existence of several leucines possessing the same chemical constitution, but which are, however, 'physically isomeric.' Thus Zollikofer[1] found that 1 part of leucine obtained from *lig. nuchae* is soluble in 27 parts of cold water. B. Gmelin[2] found that 1 part of leucine prepared from casein is soluble in 29 parts of water at the temperature of 19° C., and in 14·3 parts of boiling water, whilst 1 part of leucine prepared from haemoglobin requires 45·8 parts of water at 19° C. to dissolve it, and 18·7 parts of boiling water.

Leucine obtained by the synthetic process described at page 238 requires 117 parts of water at the temperature of 12° C. to dissolve it[3]. Leucines of approximately the same sparing solubility result from the decomposition of various proteids by Schützenberger's method[4].

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[1] Zollikofer, 'Beiträge zur Kenntniss d. elastisch. Gewebes.' *Ann. d. Chem. u. Pharm.*, Vol. LXXXII. (1852), p. 162—180.

[2] B. Gmelin, *op. cit.* p. 30.

[3] Hüfner, *Journ. f. prakt. Chemie* (2), 1, 6.

[4] Refer to the memoirs of Schulze referred to at page 241.

Leucine is, when pure, soluble in approximately 1040 parts of absolute (98 per cent.) alcohol at ordinary temperatures, and 800 parts of boiling alcohol of the same strength (Zollikofer, B. Gmelin). Leucine is insoluble in ether. It is dissolved by alkalies and acids. Impure leucine is, however, much more soluble both in water and alcohol than the pure substance.

Rotatory power. Leucine which is the product of the action of trypsin on albuminous and albuminoid bodies, or which is obtained by decomposing the proteids by means of acids, is dextrogyrous. Its specific rotatory power (α) D = + 17·3 (Schulze and Bosshard[2]). In a remarkable investigation, Professor Schulze, with whom were associated E. Bosshard and (in a great part of the work) J. Barbieri[1, 2], discovered, in the first place, that leucine which is obtained by the action of barium hydrate on the proteids at high temperatures (150°—160° C.) is inactive. They next proved that when normal, optically active leucine is heated with barium hydrate to 150°—160° it acquires the properties of the body obtained by the action of the same reagent acting on the proteids at the same temperature. The optically inactive leucine was found to be less soluble in water than normal leucine, requiring about 100 parts of water at ordinary temperatures for solution.

The next remarkable discovery consisted in proving that when *Penicillium glaucum* is sown in a suitable sterilised culture fluid to which optically inactive leucine has been added, and the organism allowed to develope for some weeks, an optically active leucine is at last found in solution, but this differs from the normal leucine in being lævogyrous. Whilst for normal leucine (α) D = + 17·3, for leucine formed under the action of *Penicillium glaucum* (α) D = − 17·5.

Reasoning by analogy, we should assume that under the influence of a high temperature two physically isomeric leucines are formed, one of which (the normal) is dextrogyrous, and the other lævogyrous; further, that the mould which effected the wonderful transformation consumed one of the isomers, viz. the dextrogyrous leucine, leaving its lævogyrous fellow untouched. In accordance with these probable assumptions, Schulze and Bosshard found that the amount of lævogyrous leucine recovered amounted approximately to one-half the weight of the inactive leucine which had been acted upon by the Penicillium, and, further, that after the process had been approximately completed, so that but very little inactive leucine could be present in the solution, freshly introduced Penicillium developed very scantily.

It will be seen in the sequel that other amido-acids possess, like

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[1] E. Schulze (unter Betheiligung von J. Barbieri und E. Bosshard ausgeführt), 'Untersuchungen über die Amidosäuren, welche bei der Zersetzung der Eiweissstoffe durch Salzsäure entstehen,' *Zeitschrift f. physiol. Chem.*, Vol. IX. (1885), pp. 63—144.

[2] Schulze and E. Bosshard, 'Untersuchungen über die Amidosäuren, &c.' *Zeitschrift f. phys. Chem.*, Vol. X. (1886), pp. 134—145.

leucine, dextro- and lævo-gyrous isomers, which are produced under the influence of high temperatures, and that the action of *Penicillium glaucum* is the same in all these cases.

Compounds of leucine with other bodies.

Leucine forms crystalline compounds with sulphuric, hydrochloric and nitric acids. The hydrochloric acid compound is represented by the formula

$$C_6H_{13}NO_2 . HCl.$$

Leucine forms two crystalline compounds with cupric oxide.

1. The compound, already referred to, which is formed when solutions of leucine are boiled with freshly precipitated cupric hydrate, separates in the form of sparingly soluble light blue rhombic tables, and has a composition represented by the formula $Cu(C_6H_{12}NO_2)_2$.[1]

2. When to a boiling solution of leucine there is added a boiling solution of cupric acetate, there separate deep blue shining crystals containing between 25·25, and 26·91 per cent. of CuO, a percentage which agrees best with the empirical formula

$$7\,C_6H_{13}NO_2 + 4\,CuO.$$[2]

Reactions which serve to identify leucine.

1. It has already been said that when leucine is heated to 170° C. it melts and volatilises unchanged. If further heated it yields amylamine and CO_2, the odour of the former being distinctly recognizable.

$$\underbrace{C_6H_{11}(NH_2)O_2}_{\text{Leucine.}} \rightarrow \underbrace{C_5H_{11}(NH_2)}_{\text{Amylamine.}} + CO_2.$$

2. The crystalline structure of leucine aids greatly in its recognition. It will be remembered that when separating from extracts of animal organs it presents the appearance of nodules and spheres; that after being sublimed it occurs in the form of plates arranged in rosettes (p. 239).

3. When leucine is treated with nitric acid and is slowly evaporated on a slip of platinum foil it yields an almost colourless residue; when one or two drops of solution of sodium hydrate are added to the residue and a little heat is applied an oily globule is formed which rolls upon the foil without wetting it (Scherer's reaction).

4. Some of the substance may be dissolved in boiling water and treated with boiling solution of cupric acetate, when the crystalline copper compound, already described, will form.

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[1] Fr. Hofmeister, 'Zur Kenntniss der Amidosäuren,' Maly's *Jahresbericht*, Vol. VII. (1878), pp. 78—80.

[2] H. Ritthausen und A. Kreusler, 'Ueber Leucin,' *Journ. f. prakt. Chemie*, 1871, p. 307.

Results of the action of HI and HNO_2 on leucine.

1. By heating leucine obtained from proteids with fuming HI in sealed tubes at from 140°—150° C. Hüfner found that there were obtained caproic acid, iodide of ammonium and iodine, according to the following equation:

$$\underbrace{C_5H_{10}(NH_2).CO(OH)}_{\text{Leucine.}} + 3HI = \underbrace{C_5H_{11}.CO(OH)}_{\text{Caproic acid.}} + NH_4I + I_2.$$

2. When leucine is dissolved in nitric acid and the solution is treated with nitrous acid, it exhibits the general reaction of the amido-acids, the whole of its nitrogen being resolved in the gaseous form and leucic (or oxycaproic) acid being formed.

$$C_6H_{11}(NH_2)O_2 + HNO_2 = \underbrace{C_6H_{11}(OH)O_2}_{\text{Leucic acid}} + N_2 + H_2O.$$

Leucic acid bears to leucine the same relation which glycollic acid bears to glycocine and lactic acid to alanin.

The relationship is shewn below:—

Glycocine	$CH_2(NH_2).COOH$	Glycollic acid $CH_2(OH).COOH$.
Alanin	$C_2H_4(NH_2).COOH$	Lactic acid $C_2H_4(OH).COOH$.
Leucine	$C_5H_{10}(NH_2).COOH$	Leucic acid $C_5H_{10}(OH).COOH$.

Isomers of Leucine.

Of all the theoretically possible leucines having the general formula $C_nH_{2n+1}NO_2$, the dextrogyrous leucine (α-amido-isobutylacetic acid) is, as already stated, the one formed in the processes of the economy. Allusion has been made to an inactive leucine which is formed under the influence of barium hydrate at high temperatures, and of a lævogyrous leucine which is presumably associated with an exactly equal quantity of the dextrogyrous body, in inactive leucine. These leucines all have the same chemical constitution as the normal leucine, being physical isomers of the latter body. The same remark applies, almost certainly, to a leucine described by Nencki. This observer[1] separated, from decomposing pancreas, a leucine which is an isomer of normal leucine, but which differs from it in certain important properties. One part requires 43·6 parts of water at 14·5° C. to dissolve it; its solubility is therefore about half that of normal leucine. It possesses a feebly sweet taste, and when heated to 210° sublimes without previous melting; under the action of heat it gives off the odour of amylamine. No details are given as to the optical properties of this leucine.

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[1] Nencki, 'Zur Kenntniss der Leucine,' *Journ. f. prakt. Chem.*, N. F. Vol. xv. 390—398.

Amido-valerianic acid, $C_5H_{11}NO_2$

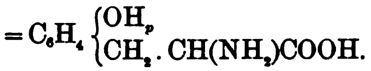
$$(C_5H_9(NH_2)O_2 = CH_2(NH_2) - CH_2 - CH_2 - CH_2 - CO.OH).$$

Occurrence. This amido-acid was found, on one occasion, in the pancreas of the ox by v. Gorup-Besanez[1], who afterwards assumed, though doubtless incorrectly, that Nencki's sparingly soluble leucine is amido-valerianic acid[2]. The two bodies have many chemical and physical properties in common, *e.g.* the compounds they form, their volatility and the sublimate which they yield. Amido-valerianic acid is, however, less soluble in water than leucine, and is optically inactive.

It appears probable that ornithin (Jaffé[3]), a base excreted in combination with benzoic acid when the latter is administered to hens, is a diamido-valerianic acid (Jaffé, Drechsel[4]). This fact renders a search for amido-valerianic acid in the products of pancreatic digestion both interesting and desirable[5].

Tyrosine $(C_9H_{11}NO_3)$[6].

Paroxyphenyl-a-amidopropionic acid



$$= C_6H_4 \left\{ \begin{array}{l} OH_p \\ CH_2 . CH(NH_2)COOH. \end{array} \right.$$

Occurrence in the organism. Tyrosine is probably never a constituent of the healthy living tissues or organs of man and the higher animals, but when found there is either the result of putrefactive decomposition or of the action of trypsin on proteid bodies, or it is the result of morbid processes.

The statement that tyrosine is probably never a constituent of the healthy organs of the higher animals requires some explanation. Until the researches of Kühne and his pupil Radziejewski[7] had proved the contrary, it was believed on the strength of the analyses of the dead pancreas made by Scherer, Gorup-Besanez and others, that this organ contains an abundance of tyrosine as one of its normal proximate principles. Ever since these researches were made the error has from time to time been repeated and it cannot, therefore, be too emphatically asserted that the pancreas during life contains only a very small quantity of leucine and no tyrosine[1]. Both these substances, as we have already stated, usually occur in large quantities in the dead pancreas, because of the rapid auto-digestion of the organ, a process which commences very shortly after death. It is only in the intestinal canal that tyrosine is a normal constituent; there it arises as one of the products of the action of trypsin on some portion of the hemipeptone which is the result of gastric and pancreatic proteolysis.

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[1] v. Gorup-Besanez, 'Ein dem Leucin homologer Körper. Bestandtheil der Bauchspeicheldrüse,' *Ann. d. Chem. u. Pharm.*, Vol. XCVIII. (1856), p. 15.

[2] v. Gorup-Besanez, *Lehrbuch d. physiolog. Chemie*, 4te Auflage, Braunschweig, 1878, see p. 223.

[3] Jaffe, *Ber. d. deutsch. chem. Gesellsch*, Vol. X. p. 1925 and XI. p. 406.

[4] Drechsel, Hermann's *Handbuch*, Vol. V. I. pp. 518, 519.

[5] Since the above was written Dr Max Siegfried's memoir, 'Ueber die chemischen Eigenschaften des reticulirten Gewebes' (*Habilitationsschrift*, Leipzig, Dec. 1892), has appeared. He has found that when adenoid tissue, which is absolutely unacted upon by trypsin, is subjected to the action of stannous chloride and hydrochloric acid it yields as a chief product of decomposition amido-valerianic acid, besides ammonia, lysine and lysatinine, but neither leucine nor tyrosine. This discovery emphasises the necessity of the search recommended in the text (Jan. 13, 1893).

[6] The name Tyrosine is derived from *τυρός*, cheese, and was given to it by its discoverer, Liebig, who first obtained it by fusing cheese with caustic potash.

[7] Radziejewski, 'Das Vorkommen von Leucin und Tyrosin im normalen Körper,' *Virchow's Archiv*, Vol. XXXVI. (1866), pp. 1—14.

Tyrosine occurs as a regular constituent in many invertebrate animals, especially in arthropoda. It was found by Warren de La Rue to be an abundant constituent of the cochineal insect (*Coccus cacti*). When thus found it is always associated with leucine.

Tyrosine has been found (first by Frerichs), together with leucine, in considerable quantities, in the liver, blood and urine of cases of acute yellow atrophy and of acute phosphorus poisoning. In smaller quantities, in cases of cirrhosis of the liver, and in the liver in severe cases of typhoid fever and of small-pox. It has been found in purulent sputa[2], in the enlarged spleen in cases of leukæmia[3], and has been described as a constant constituent of the epidermal scales in pellagra[4].

Modes of preparation of tyrosine.

Inasmuch as tyrosine is, with few exceptions, associated in its origin with leucine, its modes of preparation have already been described, as well as the methods which may be employed in separating it from leucine (see pp. 234 and 236). It only remains therefore to note certain exceptional cases in which by the decomposition of the albuminous or albuminoid bodies, tyrosine is not obtained, and to give such information as to the yield of tyrosine from various albuminous and albuminoid bodies as was previously given in reference to leucine.

In general, whatever the proteid acted upon by such reagents as dilute sulphuric acid, barium hydrate in aqueous solution at 150°—160° C. &c., the products obtained are the same in kind; nevertheless the amount of each product yielded by different substances may, *cæteris paribus*, exhibit wide differences. Whichever the albuminous or albuminoid body acted upon (with the single exception of the newly-separated '*reticulin*') we find leucine amongst the products of decomposition, though how greatly the yield of leucine may vary is stated at page 237. Tyrosine stands in this respect in a different relation to leucine, in that some of the derivatives of albumin do not yield it. The first example in illustration of such bodies is offered by gelatin. When long boiled with dilute sulphuric acid, it yields large quantities of amido-acetic acid, or glycocoll ($C_2H_5NO_2 = CH_2(NH_2)COOH$), an amido-acid which is not, in general, yielded by a similar treatment of proteids, or their derivatives and *inter alia*, it yields about 1·5 per cent. of leucine (Nencki). From gelatin, however, we can obtain no trace of tyrosine. Under the influence of putrefactive bacteria gelatin again yields glycocoll and leucine but neither tyrosine nor indol. Gelatin, it may be noted in this connection, does not, when pure, exhibit Millon's reaction. This reaction, which is furnished by all the albuminous bodies proper and by nearly all their immediate allies the albuminoid bodies, which are capable of yielding tyrosine, is identical with Hoffmann's tyrosine-reaction. It has already been stated (p. 228) that whilst solutions of amphopeptones give, in a characteristic manner, Millon's reaction, pure antipeptone fails to do so; in accordance with this fact, which they discovered, Kühne and Chittenden found that by long boiling with dilute sulphuric acid, antipeptone yields no tyrosine.

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[1] Kühne, 'Erfahrungen und Bemerkungen über Enzyme und Fermente,' *Untersuchungen aus dem Physiologischen Institute der Universität Heidelberg*, Vol. I. p. 317.

[2] Fridreich, *Virchow's Archiv*, Vol. XXX. p. 381.

[3] Salomon, 'Zur Lehre von der Leukaemie,' *Archiv f. Anat. u. Physiologie*, 1876, p. 762. Huber, 'Tyrosin und sein Vorkommen im thierischen Organismus,' *Archiv f. Heilkunde*, Vol. XVIII. (1877), p. 485.

[4] Schnitzer, quoted by v. Gorup-Besanez, *Lehrbuch d. phys. Chem.* p. 227.

Quantities of tyrosine obtained from albuminous and albuminoid bodies.

From horn, Städeler obtained 4 per cent. of its weight of tyrosine. Ellenmeyer and Schöffer obtained from *ligament. nuchae* 0·25 per cent.: from horn 3·6 per cent.: from blood-fibrin 2·0 per cent.: from egg-albumin 1·0 per cent.

Schützenberger by the action of barium hydrate, at temperatures from 160°—200° C., for a period of from 4 to 6 days, obtained from blood-albumin and vegetable-fibrin 2 per cent.: from blood-fibrin 3·3 per cent.: from casein 4·1 per cent. of tyrosine[1].

Quantities of tyrosine (as well as leucine) obtained by digestion of fibrin by trypsin.

In the paper in which Kühne first announced his discovery of the profound decomposition which the proteid molecule undergoes under the influence of the proteolytic enzyme of the pancreas, he gave details of an experiment which furnishes some idea of the quantities of leucine and tyrosine obtained by the digestion of blood-fibrin.

The fibrin had been thoroughly boiled, and then freed mechanically from much of its adherent moisture. The amount subjected to digestion was equivalent to 382 grammes of dry fibrin. It was suspended in 6 litres of water at 40°—48°, and to it was added 55 grammes of minced pancreas, corresponding to 15·2 grammes of dried gland. After a digestion lasting 6 hours, 16·0 grammes of undissolved substance remained, whilst from the solution were separated 42·5 grammes of coagulated albumin and albuminate. By subtracting the amount of the undissolved matter as well as the mass of the coagulated albumin and albuminate from the total quantity of water free fibrin and gland we obtain the amount of the substances in solution (382 + 15·2) − (11·0 + 42·5) = 343·7 grammes. Of these, 211·2 grammes consisted of peptone, 13·3 grammes of tyrosine, and 31·6 grammes of leucine. If we calculate the percentage of tyrosin and leucine obtained, in terms of the water-free proteid matter submitted to digestion (397·2 grammes), we find that the yield of tyrosine amounted to 3·3, and of leucine to 7·9 per cent. We have stated (see p. 246) that by treating blood-fibrin by the sulphuric acid method, Erlenmeyer and Schöffer obtained only 2 per cent. of tyrosine, though the yield of leucine was 14 per cent. By his barium hydrate method, Schützenberger obtained from blood-fibrin 3·3 per cent. of its weight of tyrosine. From a comparison of the results, we must conclude that the yield of tyrosine when blood-fibrin is subjected to the action of trypsin is larger than it is by either sulphuric acid or the barium hydrate method. The comparative smallness of the yield of leucine as compared with that of tyrosine probably depended upon the greater difficulty of separating quantitatively the more soluble proximate principle.

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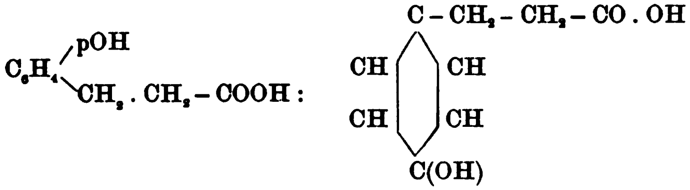
[1] All the data given under this head are taken from Maly, *Hermann's Handbuch*, Vol. v. II. pp. 218 and 219.

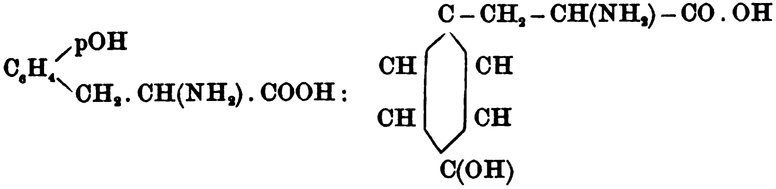
Constitution of tyrosine. Tyrosine is an aromatic compound. On many grounds it has come to be generally looked upon as derived from one of the three oxyphenylpropionic acids, viz. from the one which is designated para or p-oxyphenylpropionic acid. This relation will be rendered obvious by the following constitutional and graphic formulæ.

1. Para-oxyphenyl-*a*-propionic acid.



$$C_6H_4\left\langle\begin{matrix}pOH\\ CH_2 . CH_2 - COOH :\end{matrix}\right.$$

If we now replace an H, by NH_2-, in a CH_2 in the side chain of paroxyphenylpropionic acid, we obtain *paroxyphenyl-a-amidopropionic* acid, *i.e.* tyrosine, thus :



$$C_6H_4\left\langle\begin{matrix}pOH\\ CH_2 . CH(NH_2) . COOH :\end{matrix}\right.$$

It was formerly believed that tyrosine was a derivative of salicylic acid[1]. V. Barth, however, in 1865, shewed that when it is fused with caustic alkalies, it yields, instead of salicylic acid, its isomer paroxybenzoic acid, besides acetic acid and ammonia, thus:

$$C_9H_{11}NO_3 + H_2O + O = C_7H_6O_3 + C_2H_4O_2 + NH_3.$$

V. Barth, thereupon, advanced as a probable theory, that tyrosine might be considered as ethylamidoparoxybenzoic acid $CH_3.(NHC_2H_5).OH.CO.OH$. He subsequently, however, advanced the view that tyrosine is a para-oxyphenylamidopropionic acid[2]; this opinion has received confirmation both from Beilstein and Kuhlberg, and from Erlenmeyer and Lipp[3].

Under the influence of putrefactive processes tyrosine yields primarily para-hydrocumaric acid (paroxyphenylpropionic acid), $HO.C_6H_4.CH_2.CH_2.CO.OH$, and secondarily by the decomposition of the latter body, paroxyphenylacetic acid: $HO.C_6H_4.CH_2.CO.OH$, besides parakresol.

The following equations exhibit, according to Baumann, the successive processes of decomposition and oxidation of tyrosine[4].

$$(1)\quad \underbrace{HO.C_6H_4.CH_2.CH(NH_2).CO.OH}_{\text{Paroxyphenylamido-propionic acid, or Tyrosin}} + H_2 =$$

$$NH_3 + \underbrace{HO.C_6H_4.CH_2.CH_2.CO.OH.}_{\text{Hydroparacumaric acid}}$$

$$(2)\quad HO.C_6H_4.CH_2.CH_2.CO.OH = CO_2 + \underbrace{HO.C_6H_4.CH_2.CH_3.}_{\text{Paraethylphenol}}$$

$$(3)\quad HO.C_6H_4.CH_2.CH_3 + O_2 = H_2O + \underbrace{HO.C_6H_4.CH_2.CO.OH.}_{\text{Paroxyphenylacetic acid}}$$

$$(4)\quad HO.C_6H_4.CH_2.CO.OH = CO_2 + \underbrace{HO.C_6H_4.CH_3.}_{\text{Parakresol}}$$

$$(5)\quad HO.C_6H_4.CH_3 + O_2 = H_2O + \underbrace{HO.C_6H_4.CO.OH.}_{\text{Paroxybenzoic acid}}$$

$$(6)\quad HO.C_6H_4.CO.OH = CO_2 + \underbrace{HO.C_6H_5.}_{\text{Phenol}}$$

Physical and Chemical Properties of Tyrosine.

Crystalline form. Tyrosine crystallises in fine needles, which occur singly as well as in characteristic double broom-like bundles and in rosettes.

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[1] Odling, *Lectures on Animal Chemistry*, London, 1866, see p. 125.

[2] Barth, *Ann. d. Chem. u. Pharm.*, Vol. CLI. p. 100.

[3] Erlenmeyer u. Lipp, 'Ueber künstliches Tyrosin,' *Ber. d. deutsch. chem. Gesellschaft*, Vol. XV. p. 1544.

[4] Baumann, *Berichte d. deutsch. chem. Gesellsch*, Vol. XII. p. 1453. Drechsel, 'Chemie der Absonderungen und der Gewebe.' Hermann's *Handbuch*, Vol. V. I.

From very impure solutions it separates in part or wholly in nodules and balls very similar to those of leucine.

Crystallisations of pure tyrosine often present, to the naked eye, a white opaque paper-like appearance due to the aggregation of crystals.

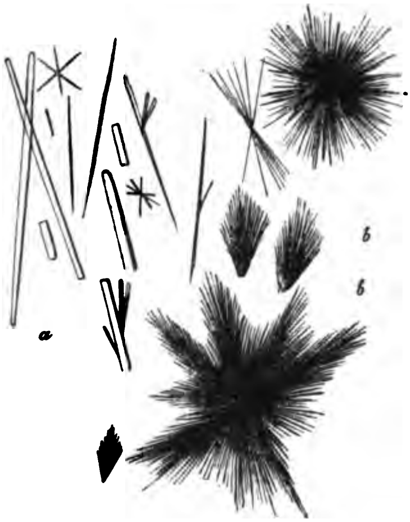

FIG. 16. CRYSTALS OF TYROSINE.

Solubility. Tyrosine is soluble in about 1900 parts of water at ordinary temperature, and in 150 parts of boiling water; it is insoluble in alcohol and ether.

Tyrosine is readily soluble in solutions of ammonia, in solutions of the caustic alkalies and their carbonates. It is likewise soluble in dilute concentrated mineral acids, with which it forms somewhat unstable compounds.

Compounds of tyrosine. Tyrosine forms a compound with hydrochloric acid, $C_9H_{11}NO_3 . HCl + 2H_2O$, which is easily soluble in absolute alcohol, but is split up by water into tyrosine and hydrochloric acid. Similar compounds with nitric and sulphuric acids have been obtained.

Compounds of tyrosine with sodium, calcium, barium, silver and mercury exist and have been more or less completely investigated[1].

The compound of copper with tyrosine deserves special mention, on account of its sparing solubility. It is obtained by boiling solutions of tyrosine with freshly precipitated cupric hydrate, and separates in the form of small, dark blue, shining needles which are soluble in 1230 parts of cold and 240 parts of boiling water, but are insoluble in alcohol and ether. It is decomposed by evaporating its solutions. It has the composition represented by the formula $(C_9H_{10}NO_3)_2\ Cu$[1]. In the detection of tyrosine the observer is much aided by the study of the crystalline form, as well as of the solubility in various agents. In addition, however, several reactions are available.

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[1] Consult *Lehrbuch der Zoochemie* von Prof. Karl B. Hofmann, Wien, 1879, p. 15.

Reinhold Hoffmann's reaction[2].

Tyrosine forms several compounds with mercury; all these when heated with a solution containing nitrous acid are coloured red. Millon's reagent is employed in testing for tyrosine. When added to solutions of this body it produces a precipitate and, on boiling, the liquid assumes a red colour, which increases in depth as the boiling is continued. Millon's reaction for the detection of proteid bodies and their derivatives depends upon the action exerted by mercuric salts, in the presence of nitrous acid upon the tyrosine residue which they contain. Nevertheless, no solution of a proteid, however concentrated, exhibits the progressively increasing colour—commencing with a pink shade and passing into a deep crimson—which is seen when solutions of tyrosine are boiled with Millon's reagent. Maly recommends that the liquid to be tested for tyrosine be first treated with a not too acid solution of mercuric nitrate, and then either with a little nitric acid, containing nitrous acid, which is diluted before being added, or with a nitrite.

Piria's reaction.

A small quantity of tyrosine is placed on a watch-glass together with one or two drops of concentrated sulphuric acid and is heated on the water bath to 50° C. After half-an-hour the solution is diluted with a little water and neutralised by means of barium carbonate. On filtering and adding to the filtrate a dilute solution of ferric chloride, free from acid, a violet colour developes. An excess of ferric chloride decolourises the solution. When, besides tyrosine, large quantities of leucine are present, Piria's reaction is interfered with.

Piria's reaction depends upon the formation of compounds of tyrosine and sulphuric acid.

Scherer's reaction.

When tyrosine is treated with a mixture of 1 part of concentrated nitric acid and 1 part of water and the mixture is evaporated to dryness a deep yellow residue is obtained, which on being moistened and warmed with sodium hydrate solution assumes at first a yellow and afterwards a deep reddish-yellow colour. Scherer's reaction depends upon the formation of nitrate of nitro-tyrosine $C_9H_{10}(NO_2)NO_3 \,.\, HNO_3$.

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[1] Franz Hofmeister, 'Zur Kenntniss der Amidosäuren.' Maly's *Jahresbericht*, Vol. VII. (1878), pp. 79 and 80. The original paper appeared in the *Sitzungsber. der Wien. Akad.* (1877), but the author has been unable to consult it.

[2] Reinh. Hofmann, 'Reaction auf Leucin und Tyrosin,' *Ann. d. Chemie, &c.* Vol. LXXXVII., p. 123.

Aspartic acid: $C_4H_7NO_4$.

(*Amido-succinic acid,* $C_2H_3(NH_2)(CO.OH)_2$.)

Occurrence. Aspartic acid was found by Ritthausen and Kreusler[1], together with glutamic acid and leucine, as a constant product, amongst the substances resulting from the decomposition of vegetable albuminous substances when these are boiled with dilute sulphuric acid.

H. Hlasiwetz and J. Habermann[2], in their first great research on the products of decomposition of the proteids, shewed that aspartic acid likewise results from the splitting-up of animal albuminous substances, under the influence of bromine. From 100 parts of dry egg albumin they obtained 23·8 parts of aspartic acid.

In their second investigation, Hlasiwetz and Habermann[3] employed hydrochloric acid and stannous chloride as decomposing agents, and again obtained aspartic acid among the products.

It was Radziejewski and Salkowski[4] who first discovered aspartic acid amongst the products of the pancreatic digestion of fibrin. V. Knieriem afterwards obtained it together with leucine and tyrosine amongst the products of the pancreatic digestion of the gluten of wheat[5].

Method of separation and identification. Aspartic acid is found in the mother liquors from which leucine and tyrosine have crystallised out. These may be further concentrated, or treated with a little alcohol, when after some time new crystalline crusts will separate. These should be dissolved in water, and the solution boiled with freshly precipitated cupric hydrate. On filtering, the blue solution will deposit the Cu-compound of aspartic acid, which has the composition $C_4H_5CuNO_4 . 4\frac{1}{2}H_2O$; this body occurs in light blue needles. It should be dissolved in HCl, and decomposed by means of H_2S, when white crystalline platelets of aspartic acid will separate out. The crystalline copper composed should again be formed, the amount of copper determined, and, if possible, an elementary analysis made. The compound of aspartic acid with copper contains 23·02 per cent. of Cu.

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[1] H. Ritthausen und U. Kreusler, 'Verbreitung der Asparaginsäure und Glutaminsäure unter den Zersetzungs-producten der Proteinstoffe,' *Journ. f. prakt. Chem.* III. (1871), p. 314.

[2] H. Hlasiwetz and J. Habermann, 'Ueber die Proteinstoffe,' *Annalen der Chemie*, Vol. CLIX. (1871), pp. 304—333.

[3] Hlasiwetz and Habermann, 'Ueber die Proteinstoffe II.' *Annalen d. Chem. &c.* Vol. CLXIX. (1873), p. 150.

[4] S. Radziejewski and E. Salkowski, 'Bildung von Asparaginsäure bei der Pancreas-Verdauung,' *Ber. d. d. chem. Gesellschaft*, Vol. VII. p. 1050.

[5] W. v. Knieriem, 'Asparaginsäure ein Product der künstlichen Verdauung von Kleber durch die Pancreas-Drüse,' *Zeitschrift f. Biologie*, Vol. XI. (1875), 198.

Physical and Chemical Properties.

Aspartic acid crystallises in small white plates.

Solubility. It is soluble with difficulty in cold water and insoluble in alcohol.

Rotatory power. Its aqueous solution is lævo-rotatory; its strongly acid solutions are dextro-rotatory and its alkaline solutions are lævo-rotatory (Pasteur, Ritthausen)[1]. A nitric acid solution of aspartic acid has a specific rotation $(\alpha)_D = +25^\circ{\cdot}16$ (Landolt). Inactive aspartic acid is obtained when the normal acid is heated to 170°—180° with hydrochloric acid (A. Michael and J. Wing)[2].

Glutamic acid $C_5H_9NO_4$.

(*Amido-pyrotarturic acid* $C_3H_5(NH_2)(COOH)_2$.)

Occurrence. Glutamic acid, as has been already said, was found by Ritthausen and Kreusler amongst the products of the decomposition of vegetable albuminous substances. Kreusler[3] having failed to obtain it by the decomposition of proteids of animal origin, advanced the view that it was one of the products which distinguished them from the kindred bodies of the vegetable kingdom. Hlasiwetz and Habermann[4], however, shewed, first of all in the case of casein and then in that of other animal proteids, that when decomposed by means of stannous chloride and hydrochloric acid, glutamic acid is formed. From casein they obtained by their method 29 per cent. of glutamic acid.

By the decomposition of "reticulin," the new phosphorus-containing proteid, which he has found to be distinctive of adenoid tissue, Siegfried has obtained a small quantity of glutamic acid[5].

Method of separation and identification. The reader is referred for detailed information as to the methods of separating glutamic acid from the other amido-acids which always accompany it, to the already quoted memoirs of Hlasiwetz and Habermann, Ritthausen, and Siegfried. Either the sparingly soluble compound, which, like the other amido-acids, glutamic acid forms with copper must be separated, purified, and decomposed, or advantage may be taken of the sparing solubility of the hydrochloric acid compounds of glutamic acid in strong HCl. With this object, the concentrated mother liquor in which the acid is suspected to exist is saturated with gaseous HCl, at the temperature of 0° C. and is placed in a freezing mixture for some hours. The HCl-salt crystallises in the form of large triclinic tables and prisms, which have the composition $C_5H_9NO_4 + HCl$, which are very sparingly soluble in cold HCl, and very soluble in water. To obtain glutamic acid from this compound, the latter is dissolved in hot water, and to the boiling solution freshly precipitated moist silver oxide is added as long as a precipitate of chloride of silver separates. The filtrate from the precipitate is treated with H_2S, decolourised, concentrated, and set aside for some days to crystallise[1].

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[1] Landolt, 'Das Optische Drehungsvermögen Organischen Substanzen.' Braunschweig, 1879. See p. 222.

[2] A. Michael and J. Wing, *Berichte d. deutsch. chem. Gesellschaft*, Vol. XVII. p. 2984.

[3] Kreusler, *Journ. f. prakt. Chemie*, Vol. CVII. p. 240.

[4] Hlasiwetz und Habermann, 'Ueber die Proteinstoffe II.' *Ann. d. Chemie, &c.* Vol. CLXIX. p. 150.

[5] Dr Max Siegfried, 'Ueber die Chemischen Eigenschaften des Reticulirten Gewebes,' *Habilitationschrift*, Leipzig, Dec. 1892.

Physical and Chemical Properties.

Glutamic acid crystallises in the form of small plates or of rhombic tetrahedra or octahedra. It is insoluble in alcohol or ether, and is sparingly soluble in cold water (at 16° C., 1 part is soluble in 100 parts of water). Its melting point is 135°—140°. Even dilute solutions reduce Fehling's solution.

Rotatory power. Aqueous and acid solutions of glutamic acid are dextro-rotatory. In the case of a dilute hydrochloric acid solution containing 9·0 grammes of HCl, and 5 grammes of glutamic acid in 100 C.C., the specific rotatory power $(\alpha)_D = +31{\cdot}1$ to $31{\cdot}6$ (Schulze and Bosshard).

In the case of glutamic acid, as in that of leucine, Schultze and Bosshard found that the dextrogyrous body, by long heating with barium hydrate at 150°—160°, yielded an inactive body of the same composition. Here, again, by sewing *Penicillium glaucum* in an appropriate nutrient sterilised medium to which the inactive body had been added, they succeeded in obtaining an optically active amido-acid, which, however, instead of being dextrogyrous, as the normal acid, is lævogyrous. Under the conditions of acidity and temperature previously referred to, the lævorotatory power of this glutamic acid $(\alpha)_D = -33{\cdot}3$ to $-33{\cdot}2$.

The slight difference between the rotation of the plan of polarisation of the dextro- and the laevo-gyrous bodies depends doubtless upon slight and unavoidable impurities.

The Cu-compound of glutamic acid obtained by boiling it with freshly precipitated $Cu(OH)_2$ separates in the form of prisms, having the colour of *lapis lazuli*, and the composition $C_5H_7NO_4Cu + 2\frac{1}{2}H_2O$ (Ritthausen, Franz Hofmeister). This salt is soluble in about 3400 parts of cold and 400 parts of boiling water.

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[1] Karl B. Hofmann, *Lehrbuch der Zoochemie*, Wien, 1879, pp. 635—637.

SECT. 11. BASES RESULTING FROM THE DECOMPOSITION OF ALBUMINOUS SUBSTANCES BY TRYPSIN.

(*Lysine, Lysatine or Lysatinine and Ammonia.*)

1. *Lysine* $C_6H_{14}N_2O_2$.

Historical introduction. Commenting upon the results which had been obtained by Hlasiwetz and Habermann, on the one hand, by the action of stannous chloride and hydrochloric acid on the albuminous substances, and by Schützenberger, on the other, who effected the decomposition of the same bodies by $Ba(OH)_2$, Drechsel argued that both processes had this in common, that they led to the production of amido-acids and of ammonia, whilst they differed materially in that, under the influence of caustic baryta, carbonic, oxalic, and acetic acids were formed, whereas these bodies did not occur when stannous chloride and hydrochloric acid effected the decomposition[1].

Hlasiwetz and Habermann had by their method obtained leucine, tyrosine, glutamic acid and ammonia, as products of the decomposition of casein, and, in addition, a small quantity of mother liquor, from which no other crystalline bodies separated. Horbaczewski by the same method had, by the decomposition of horn, obtained 16 to 18 per cent. of the HCl compound of glutamic acid, three to five per cent of tyrosine, 15 per cent. of leucine and very small quantities of aspartic acid. If we assume, argued Drechsel[2], that in these researches only one-half of the substances actually obtained could be separated in a crystalline form, even then about 30 per cent. of the original albuminous, or albuminoid, matter acted upon would be left unaccounted for, and this deficit far exceeds the amount which could be covered by the ammonia formed in the reaction: seeing that all the products of decomposition contained nitrogen, and the amount of this element in a proteid only amounts to 16 or 17 per cent.

Reasoning in this manner, Drechsel commenced an investigation of which the object was to discover some, at least, of the products resulting from the splitting-up of the albuminous molecule which had escaped previous observers, and, in the first instance, he directed his attention to bases which might presumably be present in the mother liquors of the hydrochloric acid and stannous chloride process. The investigations which he was led to conduct in person[3] and through his pupils Ernst Fischer[1], Max Siegfried[2], T. G. Hedin[3] and T. R. Krüger[4], have led to results of the deepest interest which have already thrown great light on the important question of the origin of a part of the urea formed in the organism.

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[1] Drechsel in the article 'Eiweisskörper' in Ladenburg's *Handwörterbuch der Chemie*, Vol. III. p. 548.

[2] Drechsel, 'Der Abbau der Eiweissstoffe.' Du Bois' *Archiv*, 1891, see p. 254, 'Zur Kenntniss der Spaltungsproducte des Caseins.'

[3] E. Drechsel, "Ueber ein Spaltungsproduct des Caseins," *Ber. d. K. Sächs. Gesellschaft der Wissenschaften.* Sitz am 1 Aug. 1870. "Zur Kenntniss der Spaltungsproducte des Caseins," Du Bois' *Archiv*, 1891, pp. 254—260. 'Ueber die Bildung von Harnstoff aus Eiweiss,' *Ibid.* pp. 261—265, and *Ber. d. deutsch. chem. Gesellsch.* Vol. XXIII., p. 3096.

Pure casein was the body first investigated by Drechsel, and he subjected it to decomposition by the method employed by Hlasiwetz and Habermann, adding however to the contents of the flask metallic zinc, so as to keep up a constant evolution of hydrogen, the apparatus being arranged so as to exclude atmospheric oxygen.

Employment of phosphotungstic acid to precipitate bases.

When the decomposition of the proteids had been completed, the contents of the flask were somewhat diluted and freed from tin by the action of H_2S. Having been evaporated to the original volume, the hot solution was treated with a hot saturated solution of crystallised phosphotungstic acid[5]. This reagent produces a precipitate in which all the bases are contained, whilst the filtrate contains all the amido acids.

The precipitate was then washed with water containing five per cent. of sulphuric acid and an equal quantity of phosphotunsgstic acid, until all traces of chlorine had disappeared; it was then suspended in boiling water and decomposed by the addition of a slight excess of baryta water. The fluid having been boiled, to expel ammonia, was then filtered, and from the filtrate the excess of barium precipitated exactly by sulphuric acid. The filtrate from the barium sulphate precipitate was then saturated with HCl and concentrated on the water bath. The thick syrup thus obtained when placed over sulphuric acid gradually became converted into a crystalline mass. By treating this with spirits of wine, from the already almost solidified syrup a beautifully crystalline hydrochlorate was obtained, which was found to be readily soluble in water, but insoluble in absolute alcohol. This proved to be the hydrochlorate of the new base lysine, $CH_4N_2O_2$. In the mother liquor the hydrochlorate of lysatinine is contained. In subsequent researches the latter part of the process was somewhat modified. Thus the syrupy liquid containing the hydrochlorate of lysine was dissolved in 50 per cent. alcohol, and treated with alcoholic solution of platinum chloride. This reagent precipitated, in the first instance, some potassium platinochloride. After separation of the latter, the further addition of alcohol caused the separation of a platinochloride, which after being crystallised repeatedly, presented the appearance of beautiful yellow needles, and which had the composition, $C_6H_{14}N_2O \cdot H_2PtCl_6 + C_2H_5 \cdot OH$. The mother liquid contains the platinochloride of lysatinine, to be afterwards described. From this salt the platinum can be separated by sulphuretted hydrogen, and a crystalline hydrochlorate obtained. By boiling the latter with freshly precipitated $Pb(OH)_2$, the base is set free; it has not however been obtained in a crystalline condition.

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[1] Ernst Fischer, 'Ueber neue Spaltungsproducte des Leimes,' Du Bois' *Archiv*, pp. 265—269.

[2] Dr Max Siegfried, 'Zur Kenntniss der Spaltungsproducte der Eiweisskörper,' *Ber. d. deutschen chemischen Gesellschaft.* Vol. XXIV. p. 418, and Du Bois' *Archiv*, 1891, pp. 270—273.

[3] Dr S. G. Hedin, 'Zur Kenntniss der Producte der tryptischen Verdauung des Fibrins,' Du Bois' *Archiv*, 1891, pp. 273—278.

[4] E. Drechsel und Th. R. Krüger, 'Zur Kenntniss des Lysins,' *Ber. d. deutsch. chemisch. Gesellschaft.* Vol. XXV. (1892), p. 2454.

[5] This reagent had been long employed as a general precipitant of vegetable bases, being known as 'Scheibler's reagent'; it had been used by Körner (Pflüger's *Archiv*, Vol. II. p. 226) as a precipitant of creatinine, and had been employed by Dr Franz Hofmeister for the separation of kynuric acid from the urine of dogs; this author shewed that besides creatinine, it precipitated xanthine (F. Hofmeister, 'Ueber die durch Phosphorwolframsäure fällbaren Substanzen des Harns,' *Zeitschr. f. phys. Chem.*, Vol. V. (1881), pp. 67—74).

Another method of separating lysine, by taking advantage of the formation of a very sparingly soluble silver compound will be described under lysatinine.

Constitution and compounds of lysine.

Lysin, $C_6H_{14}N_2O_2$, has the composition of a diamido-caproic acid and is a homologue of diamido-valerianic acid, which, as has already been stated (p. 244), is presumably identical with Jaffe's ornithin. Lysine forms two compounds with HCl, having respectively the composition $C_6H_{14}N_2O_2 \cdot HCl$ and $C_6H_{14}N_2O_2 \cdot 2HCl$[1].

It is optically active (dextrogyrous) but when heated to 150° with baryta water, although it is not decomposed, it yields an optically inactive isomer, the platinochloride of which crystallises without either water or alcohol of crystallisation and has the formula $C_6H_{14}N_2O_2 \cdot H_2PtCl_6$ (Siegfried)[2].

2. *Lysatinine,* $C_6H_{11}N_3O$ (*or Lysatine* $C_6H_{13}N_3O_2$?).

In describing the methods of separating lysine we have stated that one of these consisted in the formation of the platinochloride of the base, which could be obtained in the form of beautiful needle-shaped crystals. In the mother liquors from which lysine platinochloride was separated, Drechsel discovered a second base, to which he gave the name of lysatine or lysatinine.

The interest which this base possesses in relation to the formation of urea in the organism is a sufficient reason for the somewhat lengthened treatment of Drechsel's researches on the bases which result from the decomposition of the proteid molecule.

Preparation of lysatinine.

In describing the preparation of lysine by converting it into a platinochloride, it was mentioned that the mother liquor contains the platinochloride of a second base, lysatinine. In order to obtain it, this mother liquor is considerably diluted with water and, by distillation in vacuo, freed from alcohol and ether. From the aqueous solution which remains, the platinum is separated by means of H_2S, and the filtrate heated on the water bath, to drive off free HCl, then concentrated to syrupy consistence. This syrup is diluted with water and a concentrated solution of silver nitrate added, drop by drop, from a burette, so as to free it exactly of chlorine. The filtrate and washings from the precipitated silver chloride are then again concentrated to syrupy consistence and treated with the same volume of silver nitrate solution as was previously employed to precipitate the chlorine. On now adding to this mixture five or six volumes of alcohol and some ether it becomes very turbid, and subsequently, as the turbidity disappears, a thick oil falls to the bottom of the vessel in which the precipitation is effected. The clear mother liquor is now poured off and gradually treated with ether in small quantities, until a separation of crystals, which adhere to the walls of the vessel, commences: at this stage a large excess of ether is added and the mixture set aside for the night in a cool place. The following day snow-white needles and flakes are found to have separated, which are recrystallised from a small quantity of water, to which alcohol and ether are added, and are thus obtained perfectly pure[1].

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[1] E. Drechsel and J. R. Krüger, 'Zur Kenntniss des Lysins,' *Ber. d. Deutsch. Chem. Gesellschaft*, Vol. xxv. p. 2455.

[2] M. Siegfried, 'Zur Kenntniss der Spaltungsproducte der Eiweisskörper,' *op. cit.* The specific rotation of this body has yet to be determined (May, 1893).

Siegfried's method of separating lysine and lysatinine directly from the phosphotungstic precipitate[2].

From the precipitate which phosphotungstic acid produces when added to the products of decomposition of the proteids, lysine and lysatinine may be readily separated. The precipitate having been freed from chlorine, it is dissolved (almost completely) in boiling water and decomposed by means of the smallest possible excess of $Ba(OH)_2$. The filtrate from the barium precipitate is saturated with CO_2, boiled for half-an-hour, filtered and, when cold, treated with solution of silver nitrate, so long as a precipitate falls. The voluminous precipitate is separated and thoroughly washed with water.

The filtrate from the silver precipitate is concentrated to the consistence of a thin syrup and then, little by little, treated with small quantities of absolute alcohol, which causes a somewhat dense precipitate, which assumes an obscurely crystalline form and which contains lysine. (From this precipitate the platinochloride of lysine may be obtained, in a pure condition, by decomposing by means of H_2S, concentrating the filtrate, treating with $PtCl_4$, then with alcohol and ether in the manner described at p. 255.)

The filtrate from which the smeary compound of lysine and silver has separated through the gradual addition of alcohol, is still further treated with alcohol, when the fine crystalline compound of lysatinine and silver nitrate is obtained, exactly as described previously.

Composition of the silver compound of lysatinine.

The analysis of the silver compound leads to the formula $C_6H_{13}N_3O_2 \,.\, HONO_2 + AgO \,.\, NO_2$, from which however probably is to be subtracted a molecule of water of crystallisation.

Lysatinine or lysatine?

From the empirical formula derived from the analysis of the previously described compound, lysatinine is seen to have the composition of a creatine or a creatinine, according as we assume that one molecule of water is or is not water of crystallisation. Its composition in the latter case would be expressed by the formula $C_nH_{2n+1}N_3O_2$, which is also the empirical formula of creatine, $C_nH_{2n+1}N_3O_2$; in that case we should term it *lysatine*.

Creatine ... $C_4H_9N_3O_2$.
Lysatine ... $C_6H_{13}N_3O_2$.

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[1] The details of the process are taken from Ernst Fischer's paper, 'Ueber neue Spaltungsproducte des Leimes.' Extract from 'Inaugural Dissert.,' Leipzig, 1890, in Drechsel's memoir, 'Der Abbau der Eiweissstoffe.'

[2] Siegfried, 'Zur Kenntniss der Spaltungsproducte der Eiweisskörper,' see note 2, p. 254.

Like creatine and creatinine, lysatinine decomposes with the formation of urea.

We now come to the deeply interesting, central fact resulting from Drechsel's researches.

It had long been the object of the endeavours of physiological chemists to obtain urea directly by the decomposition of albuminous substances. The French chemist Béchamp believed, indeed, that by the oxidising action of potassium permanganate he had succeeded in obtaining urea. Städeler and O. Lohr, on repeating the experiments of Béchamp, failed to confirm his discovery. In spite of the asseverations of Ritter in support of the accuracy of Béchamp's assertion, Tappeiner on going over the same ground obtained the same results as Städeler and O. Lohr. The probable source of error has been explained by F. Lorsch, who has found that under the conditions of Béchamp's experiment, with some modifications, guanidin is formed, this substance having probably been mistaken for urea by Béchamp[1].

By a process not of oxidation, but of decomposition, in which probably, secondary products of decomposition are less likely to arise than by any other, we have seen that Drechsel has obtained the base lysatinine, which has an empirical formula homologous to that of creatine or creatinine. As creatine when boiled with baryta water splits into sarcosin and urea, it occurred to Drechsel that his new base, lysatinine, would in all probability be similarly decomposed and furnish urea as a product. Experiment at once verified the anticipation. From 10 grammes of the double salt of lysatinine and nitrate of silver, by boiling (after previous separation of the silver) for 25 minutes with excess of baryta water, he obtained 1 gramme of pure urea nitrate, from which he isolated urea, which he identified by a comparison of physical and chemical properties and by a nitrogen determination. Thus it has been possible to prove that by processes of simple decomposition, in which oxidation is absolutely excluded, urea can be derived directly from the albuminous substances. The whole bearing of these discoveries will be discussed in the third volume of this work in connection with the general question of the mode of formation of urea in the body. It may here be stated, however, that Drechsel is far from believing that the whole or the major part of the urea formed in the organism is due to the decomposition of lysatinine. On the contrary, he is of opinion that presumably only about one-ninth of the urea extracted is thus derived.

Reference has been made to secondary, as distinguished from primary, products of the decomposition of proteids; the distinction will be easily made plain. The carbonic, oxalic, and acetic acids which were obtained when proteids were decomposed by long heating with $Ba(OH)_2$ at high temperatures, and which were not obtained by the non-oxidising action of $Sn\ Cl_2$ and HCl, are examples of secondary products.

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[1] The references to the original papers will be found in Drechsel's memoir, 'Ueber ein Spaltungsproduct des Caseins.'

When lysine is heated with $Ba(OH)_2$ at 150°—160° it is not split up, and can be again recovered (Siegfried). When, however, lysatinine is subjected to the same process, it is split up and barium carbonate is formed. Thus is explained the origin (at least in part) of the CO_2 which is one of the products of Schützenberger's process, and which is to be looked upon as a secondary product.

Drechsel assumes that 1 molecule of lysatinine, when decomposed with caustic baryta, yields 1 molecule of urea, which by further oxidation will yield 1 molecule of CO_2. From 100 parts of dry albumin treated by his baryta method, Schützenberger obtained a maximum quantity of 12·5 parts of $BaCO_3$, which corresponds to 2·79 parts of CO_2. These 2·79 parts of CO_2, on the assumption stated above, may have their origin in 8·95 parts of lysatinine, or 3·8 parts of urea springing from the decomposition of this body, respectively. Thus from 100 parts of dry proteid matter there probably are derived in the organism 3·8 parts of urea, by processes purely of decomposition. But how can the relation between the urea formed in this way to the total amount of urea formed by the decomposition of the organism be calculated? 100 parts of albuminous matter, in round numbers, contain 16 per cent. of nitrogen, which is almost entirely excreted as urea. The 16 per cent. of N correspond to 34·3 parts of urea. If we now compare the amount of urea which can be derived from lysatinine directly split off from the proteids, with the total amount of urea which the proteid can furnish in the economy, we arrive at the result that the proportion of the former to the latter is as 1 : 9 (3·8 : 34·3 :: 1 : 9·02).

How the other nine-tenths probably arise will be matter for discussion in the sequel.

Lysine and lysatinine actual products of digestion by trypsin.

The experiments of Drechsel, Fischer, and Siegfried had shewn that under the HCl and $SnCl_2$ process, the two bases which we have been considering are formed, however varying the nature of the albuminous body. Thus Drechsel discovered lysine and lysatinine in the products of the decomposition of casein. His pupil, Fischer, separated these bases by following the same process with gelatin; and Dr Max Siegfried obtained the same results, working with conglutin, gluten-fibrin, hemiprotein, Maly's oxyprotosulphonic acid and egg-albumin.

That the decomposition of proteids proceeds as has been stated, when they are subjected to the action of reagents which are capable of splitting them up, will appear to some to afford no sufficient ground for assuming that the same process is likely to occur in the animal economy and, until this is proved, the arguments which have been developed will seem to these objectors to be fanciful and of little value. But direct experiment is no longer wanting to prove that lysine and lysatinine are formed under conditions which necessarily must lead to their production in the alimentary canal.

Dr S. G. Hedin, working under Drechsel, separated from the products of the pancreatic digestion of 3000 grammes of moist fibrin, 28 grammes of the chemically pure platinochloride of lysine, besides enough of the silver compound of lysatinine to establish its identity. Further, from 150 grammes of Kühne's dry pancreas, Hedin obtained 0·7 grammes of the pure platinochloride of lysine, which enabled the identity of the body to be determined by elementary analysis, and doubtless lysatinin was also present[1].

Ammonia: NH_3.

When albuminous substances are split up by the action of $Ba(OH)_2$ at high temperatures (Schützenberger's process) products are obtained of which some, such as leucine, tyrosine and aspartic acid, are probably *primary*, *i.e.* are produced directly by the splitting-up of the proteid molecule, whilst others, such as acetic, oxalic and carbonic acids, are *secondary*, *i.e.* are produced either by the simple decomposition or by the oxidation of bodies which were the primary results of decomposition but are not able to remain undecomposed under the particular conditions. Thus it has been shewn that a part, if not the whole, of the carbonic acid obtained by Schützenberger must be derived from the secondary decomposition of lysatinine, itself a primary product of the splitting-up of proteids. In judging of the primary or secondary relations of these products of decomposition very great stress must be laid upon the evidence which establishes whether the body under consideration is formed, whichever the method of decomposition.

Amongst the products obtained both by Schützenberger's method of decomposition and by that followed by Hlasiwetz and Habermann, Drechsel and others ($Sn\ Cl_2$ and HCl), NH_3 occupies a constant place. It was, therefore, interesting to determine whether, under the influence of trypsin, ammonia is formed. The question has been decided in the affirmative. In an investigation made in Hoppe-Seyler's Laboratory, under conditions which exclude the probability of putrefactive changes, in trypsin digestions which lasted a very short time (4 hours), and in which the temperature was only 32° C., small quantities of NH_3 were rapidly formed[2].

Stadelmann[3], in Kühne's laboratory, repeated Hirschler's experiments under conditions which absolutely excluded the possibility of putrefaction and obtained exactly the same results.

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[1] Dr S. G. Hedin, 'Zur Kenntniss der Producte der tryptischen Verdauung des Fibrins.' In Drechsel's 'Abbau der Eiweissstoffe.'

[2] Dr August Hirschler, 'Bildung von Ammoniak bei der Pancreasverdauung von Fibrin.' (Aus dem physiologisch. chemisch. Laborat. in Strassburg.) *Zeitschrift f. phys. Chemie*, Vol. x. (1880), pp. 302—305.

[3] E. Stadelmann, 'Bildung von Ammoniak bei Pankreasverdauung von Fibrin,' *Zeitschrift f. Biologie*, Vol. xxiv. (1888), pp. 261—266.

Are Xanthine-bases products of digestion by Trypsin?

Adenine $C_5H_5N_5 + 3H_2O$. Xanthine $C_5H_4N_4O_2$.
Hypoxanthine $C_5H_4N_4O$. Guanine $C_5H_5N_5O$.

We have now, before concluding our account of the well-defined products which arise by the action of trypsin on the proteids (independently of putrefactive bacteria) to consider briefly and to examine the value of the facts which have been placed on record, and which might, at first sight, appear to establish some relation between the bases enumerated above (and often designated xanthine-bases[1]) and pancreatic digestion.

Scherer's discovery of guanine and xanthine in pancreatic tissue.

In his investigations on the bases which are contained in various tissues and organs, Scherer found small quantities of guanine and xanthine in the tissue of the pancreas of oxen[2]. The amounts do not, however, differ materially from those found in other glandular organs and fail, therefore, to establish any special connection between these bodies and pancreatic proteolysis. Chittenden, in the course of a research to be afterwards referred to, found that the pancreatic tissue contains an appreciable quantity of hypoxanthine and xanthine, but he proved that these bodies preexisted in the pancreas and were not the products of auto-digestion, as their amounts did not increase when the dried pancreatic tissue was digested at 40° C. for 24—48 hours in a weak alkaline solution.

The observations of Salomon and of Krause.

Hypoxanthine and xanthine in small quantities were stated by Salomon[3] and by Krause[4], who worked under him, to be products of the digestion of blood-fibrin by pepsin as well as by trypsin. These authors further stated that both bodies were likewise formed when fibrin is digested at the temperature of the body with weak hydrochloric acid.

Chittenden's researches.

Working under Kühne's direction, Chittenden[5] repeated Salomon's experiments. He found that when blood-fibrin is subjected to digestion either with pepsin or trypsin, small but easily recognisable quantities of the silver compound of hypoxanthine could be obtained. He likewise obtained hypoxanthine when he subjected blood-fibrin to the action of boiling water, as well as when he digested it for two or three days with a 2 p.c. sol. of hydrochloric acid at the temperature of 40° C.

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[1] Hypoxanthine and xanthine have been dealt with at length in Vol. I. (1st edition, pp. 329—332). A full description of Adenine will be found in the 2nd edition of Vol. I., in connexion with the chemistry of the Nucleins. Guanine will be described in Vol. III. In this place we shall only refer to these bodies so far as they appear to bear on pancreatic digestion.

[2] Scherer, *Annal. d. Chem. u. Pharm.*, Vol. CIX. p. 257.

[3] Salomon, 'Ueber die Verbreitung und Entstehung von Hypoxanthin und Milchsäure im thierischen Organismus,' *Zeitschr. f. phys. Chemie*, Vol. II. (1878—79), pp. 65—95; refer to p. 90.

[4] Hugo Krause, 'Ueber Darstellung von Xanthinkörpern aus Eiweiss,' Inaug. Diss. Berlin, 1878.

[5] R. H. Chittenden, 'On the Formation of Hypoxanthin from Albumin,' *Journal of Physiology*, Vol. II. (1879—80), pp. 28—37.

Chittenden, in comparative experiments, shewed that when egg-albumin was substituted for fibrin, no hypoxanthine could, with certainty, be discovered, except in the case of the pancreatic digestion of egg-albumin, in which he obtained positive results.

As will be shewn in the sequel, the cardinal fact to be made out was whether blood-fibrin contains hypoxanthine preformed or not. In the former case, Salomon's and Chittenden's results would merely prove that under the circumstances of their experiments hypoxanthine had been dissolved out of the fibrin in which it had been occluded. Chittenden believed that he had proved that fibrin does not contain hypoxanthine, by boiling it for long periods with absolute alcohol, and then examining the alcohol, which was found to be free from hypoxanthine. Drechsel[1], however, shewed that this experiment does not by any means settle the question of the absence of hypoxanthine from fibrin.

Kossel's researches on nucleins and their relations to hypoxanthine.

In the year 1879 Dr Albrecht Kossel published a research[2] on the nuclein of yeast, in which he announced that amongst the soluble products of its decomposition was a not inconsiderable quantity of hypoxanthine. In a subsequent paper[3] he shewed that when the nuclein of yeast is merely boiled with water, a quantity of hypoxanthine (which he estimated at about 1 per cent.) is formed, this body being split off from the nuclein molecule.

Extending his researches to nucleins from other sources he similarly proved that, under the same circumstances, they yield hypoxanthine. From these facts, Kossel argued that the small quantities of hypoxanthine found by Salomon and Chittenden when blood-fibrin was digested with pepsin and trypsin, were derived from the nuclein of the white blood corpuscles necessarily occluded in the fibrin, and that these experiments, therefore, in no way sufficed to prove that hypoxanthine or xanthine are products of the decomposition of proteids either by pepsin or trypsin[4].

Kossel has shewn that not only hypoxanthine but likewise xanthine and guanine result from the decomposition of nucleins.

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[1] Drechsel, 'Zur Frage nach der Entstehung von Hypoxanthin aus Eiweisskörpern,' *Ber. d. deutsch. chem. Gesellschaft*, Vol. XIII. p. 240.

[2] Dr Albrecht Kossel, 'Ueber das Nuclein der Hefe,' *Zeitschrift f. phys. Chem.*, Vol. III. (1879), p. 284—291.

[3] Dr Albrecht Kossel, 'Ueber die Herkunft des Hypoxanthins in dem Organismus,' *Zeitschrift f. phys. Chem.*, Vol. V. (1881), pp. 152—157.

[4] Salomon has adopted the view of Kossel as to the origin of hypoxanthine from nuclein: "Nachdem ich gezeigt hatte dass das Nuclein als die Quelle dieser Körper im Organismus anzusehen ist, sind alle Experimente, die man für die Bildung dieser Substanzen aus den Eiweisskörpern angeführt hat, hinfällig geworden. Salomon hat meine Beweise anerkannt und damit seine frühere Ansicht zurückgezogen." Kossel, 'Zur Chemie des Zellkerns,' *Zeitschrift für physiolog. Chemie*, Vol. VII. pp. 7—22 (see pp. 15 and 16).

Kossel's discovery of adenine.

Subsequently, Kossel found that when the pancreas is boiled for three or four hours with a very dilute sulphuric acid (one part of concentrated sulphuric acid in four parts of water) a base is set free, *adenin*, which is a polymer of HCN, and which has the composition $C_5H_5N_5 + 3H_2O$.

This base, he has shewn, is formed in the first instance from nuclein, and the hypoxanthine which is obtained by decomposing nuclein is derived from adenin[1]. When the latter base is treated with nitrous acid, hypoxanthine is formed.

$$C_5H_5N_5 + HNO_2 = C_5H_4N_4O + N_2 + H_2O.$$

Adenin appears to be an amido-hypoxanthine and to bear the same relation to it that guanine bears to xanthine.

After an examination of the facts adduced, the conclusion is inevitable that the so-called xanthine bodies do not originate by the profound decomposition of the albuminous or albuminoid bodies under the influence of trypsin, but are the products of the decomposition of nucleins, a decomposition which appears to occur with great ease under a variety of circumstances capable of effecting the hydrolytic splitting-up of organic compounds.

Tryptophan.

We shall by this name[2] designate a substance as yet known only by the reactions which it exhibits when its solutions are treated with chlorine or bromine water, but which may be found among the products of decomposition of the albuminous molecule, in whatever manner this may be brought about.

History.

Tiedemann and Gmelin[3] observed for the first time that the pancreatic juice of the dog assumes a rose-red colouration when it is treated with chlorine water, and assumed that this reaction was characteristic of the pancreatic secretion. Claude Bernard[4], however, shewed that the perfectly fresh and normal pancreatic juice does not exhibit the reaction, but that it is obtained with the juice which has been kept for some time, unless putrefaction sets in, when it ceases. Claude Bernard observed that when, through putrefaction, the chlorine reaction could no longer be obtained, if the liquid were precipitated with lead acetate, the filtrate freed from lead by dilute sulphuric acid, and the second filtrate were treated with nitric acid containing nitrous acid, a red colour was produced; this he believed to be caused by the same body which had originally caused the rose colouration with chlorine.

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[1] Kossel, 'Ueber das Adenin,' *Zeitsch. f. phys. Chem.*, Vol. x. (1886), pp. 250—264.

[2] The name Tryptophan has been suggested by Neumeister as indicating the origin of the body in the decomposition of proteids. It is derived from *θρύπτομαι*, to be broken, and *φαίνω*, to bring to light.

R. Neumeister, 'Zur Physiologie der Eiweissresorption und zur Lehre von den Peptonen,' *Zeitschrift für Biologie*, Vol. xxvii. (1890), pp. 309—373; see, concerning Tryptophan, p. 345 (note).

E. Stadelmann has applied the term Proteinochromogen to the same substance. Surely it is scarcely appropriate to apply a name implying that it is the cause of the colours displayed by the proteids (?), to a body which is but an unknown product of their decomposition.

[3] Tiedemann and Gmelin, 'Die Verdauung nach Versuchen.' Heidelberg, 1831.

[4] Claude Bernard, 'Mémoire sur le Pancréas.' *Comptes Rendus, Supplément*, i. (1856), pp. 403—409.

Kühne[1] proved this view to be untenable, the nitric and nitrous acid reactions being due to the formation of indol, which could be separated by distillation. Kühne shewed that the very different substance which exhibits the chlorine reaction also gives a red or violet colour when treated with bromine water, and since he introduced its use bromine water has generally been employed, for this purpose, in the laboratory instead of chlorine water.

It is mainly due to the researches of Kühne that we have become acquainted with the fact that tryptophan, as we shall henceforth designate it, is produced not only as one of the products of the pancreatic decomposition of proteids, but is likewise to be found (though it is rapidly destroyed and disappears) as a constant product of the splitting-up of the albuminous molecule, in whatever manner this may be brought about. Kühne is of opinion that it is one of the products of the decomposition of the hemi-moiety of the molecule.

Thus it is one of the products of decomposition when the proteids are treated by Schützenberger's process[2], as well as when they are boiled with 5 per cent. sulphuric acid, though in the latter case the reaction is slight, and the body which occasions it rapidly disappears[3].

Chemical and physical properties of solutions of tryptophan.

Tryptophan is slightly soluble in alcohol, ether, and chloroform (Krükenberg). It is more readily soluble in amyl alcohol, which may be employed to effect its separation (Hemala[4]). In the latter case the reaction should be obtained by the use of chlorine water, as bromine is soluble in amyl alcohol, and imparts to it a yellow colour.

The tinctorial intensity of tryptophan is very great, so that solutions which give an intense violet colouration with chlorine, furnish but a small quantity of the colouring matter. According to Krükenberg, chlorine or bromine do not cause the colour reaction of tryptophan by an oxidising action, but by actually entering into combination with the body. This author found that tryptophan is diffusible, and that its solutions exhibited an absorption band in the vicinity of D.

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[1] Kühne, 'Ueber Indol aus Eiweiss,' *Ber. d. deutsch. chem. Gesellschaft*, Vol. VIII. (1865), p. 206.

[2] Neumeister, 'Ueber die Reactionen der Albumosen und Peptone,' *Zeitschrift für Biologie*, Vol. XXVI. (1890), see p. 332.

[3] Neumeister, *loc. cit.* See note to page 329.

[4] Rich. Hemala, 'Zur Kenntniss der in der chemischen Physiologie zur Anwendung gekommenen Nitroprussid-reactionen' in Krukenberg's *Chemische Untersuchungen zur wissenschaftlichen Medicin*, 2tes Heft, June 1888, p. 118.

For the discussion of many facts concerning tryptophan, and for the description of painstaking methods employed in the attempted separation of the body, the reader is referred to a paper on the subject by Stadelmann[1].

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[1] E. Stadelmann, 'Ueber das beim tiefen Zerfall der Eiweisskörper entstehende Proteïnochromogen, den die Bromreaction gebenden Körper,' *Zeitschrift f. Biologie*, Vol. xxvi. (1890), pp. 491—526.

CHAPTER IV.

THE BILE.

INTRODUCTORY OBSERVATIONS.

IN the preceding chapters of this book the various secretions have been considered in connection with the general physiology of the organs which produce them, some account having also been given of the structure of these. In the case of the bile, however, a different course will be followed, for reasons which will presumably commend themselves to the readers of a book which aims at a thorough chemical treatment of the processes of the animal economy, considered, however, from the point of view of the philosophical biologist.

In this volume we shall consider the general chemical composition of the bile, its proximate principles and their transformations, and the probable part played by the bile in the intestine, independently of the general physiology of the organ which produces it.

The bile is to be looked upon as a comparatively insignificant by-product[1] resulting from the extremely complex and diverse chemical operations which have their seat in the liver, the largest and, perhaps, the most important of all the glands of the body. This by-product furnishes as little insight into the great operations which are conducted in, and by, this great chemical factory, the liver, as might be obtained of the nature and magnitude of the operations carried on in extensive chemical works, were we only permitted to study the chemical composition of a drain into which some alone of the waste products of the works were regularly discharged. Doubtless this study might throw *some* light on the operations of the factory and would, certainly, be necessary to their complete investigation, which, however, would only be possible when we were placed in a position to examine the raw materials entering, the structure and relative relations of the various parts of the factory, the nature and mutual dependence of the various chemical operations carried on within its precincts, no less than all the products resulting from them. Such a study we propose to make in the case of the liver in Book III. It cannot be undertaken until we have completed our examination of all the processes which have their seat in the alimentary canal. For the latter purpose we are, however, compelled to undertake, at the present stage, a detailed study of the bile.

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[1] 'The human liver weighs from 1500 to 2000 grms., and produces in twenty-four hours about 400 to 600 grms. of bile. The parotid, which only weighs from 24 to 30 grms., produces in the same time from 800 to 1000 grms. of secretion' (Bunge). The comparison quoted indicates how subordinate must be the part played by the bile in respect to the general functions of the liver.

SECT. 1. METHODS OF OBTAINING BILE.

In the case of the bile we do not, in the majority of animals, encounter the difficulties which present themselves in the case of the other secretions of the alimentary canal, when we desire to obtain them in a state of purity. In vertebrate animals generally, there exists in connection with the hepatic duct 'a unilateral cæcal diverticulum' the gall-bladder, in which, during the periods when a continuous flow of bile into the intestines is not required, the secretion may accumulate, and whence we usually obtain it for the purposes of chemical study and research.

That animals, such as the carnivora, in which digestion is a more or less intermittent process, should always possess a gall-bladder is intelligible enough, and that the exceptional cases in which this reservoir is absent should occur, *mainly*, in the herbivora is on the whole intelligible. It is impossible, however, to account for the exceptions, so far as the Author knows, either on morphological or physiological grounds. Thus, whilst oxen and sheep conform to the rule and have a gall-bladder, deer have none. The solidungula are distinguished by absence of gall-bladder. Whilst our domestic birds, in general, possess a gall-bladder, the pigeon has none. The parrot and the ostrich, amongst well-known birds, also have no gall-bladder.

Whilst we are able, as has been said, to obtain, for chemical study, bile from the gall-bladder of dead animals, it is impossible to study the process of secretion, to determine its relations in point of time to the various digestive acts, and to form adequate conceptions of the part which it plays in the various digestive processes, unless we supplement the facts ascertained in other ways by observing animals in which biliary fistulæ have been established.

Establishment of biliary fistulæ.

Schwann[1] was the first to establish biliary fistulæ, and his method of operating has in general been imitated by subsequent observers.

The dog is the animal which has almost invariably been employed when the object has been to make a *permanent* biliary fistula. Having been kept without food for 24 hours preceding the operation, and having been deeply narcotised, first by means of morphia and then of chloroform, an incision about two inches in length is made through the abdominal wall in the *linea alba*, commencing at the xyphoid cartilage. The gall-bladder, which is distended with bile in the fasting animal, is brought into view and, with little difficulty, the cystic is traced to the common bile-duct (ductus communis choledochus). The latter runs by the side of the portal vein and the hepatic artery. By means of a blunt aneurism needle, the common bile-duct is separated and a ligature applied, in the first instance, as near as possible to the junction of the cystic with the hepatic duct. The duct is then followed down and a second ligature applied as close as possible to the duodenum. The portion of the duct intervening between the two ligatures is cut out, with the object of preventing the re-establishment of the duct, an event which is otherwise very apt to occur, especially if any temporary hindrance to the outward flow of bile should arise.

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[1] Th. Schwann, 'Versuche um auszumitteln ob die Galle im Organismus eine für das Leben wesentliche Rolle spielt,' *Archiv f. Anat. u. Phys.* (1844), pp. 127—159. Although Schwann has the merit of having first established permanent biliary fistulæ, the dogs upon which he operated and which survived the immediate effects of the operation died in two or three hours, and he was in consequence led to attach an exaggerated and untrue importance to the part which the bile plays in the economy. Schwann employed no cannulæ to collect the bile. So far as the Author knows it was Blondlot who first did so in cases of biliary fistulæ.

This, the first and easier, part of the operation having been completed, the apex of the gall-bladder is seized, by means of artery forceps, and drawn to the upper end of the incision in the abdominal wall. Two sutures are then introduced, one at either side, very close to the apex of the gall-bladder, and these are fixed, one on one side and one on the other, to the upper end of the incision. The lower part of the latter is brought together *lege artis* by sutures, and then the very apex of the gall-bladder is incised by means of scissors. Having applied additional sutures so as to unite firmly the muscular and cutaneous edges of the incision except at the point where the gall-bladder is secured, the animal is left for a few days. The application of a shield (fitted to the animal before the operation), partly made of guttapercha and partly of metal, and which admits of being strapped across the animal's back, is of great assistance in the after treatment.

If the operation has been performed, as should invariably be the case, with the strictest antiseptic precautions, the animal usually recovers. It is, however, only in comparatively rare cases that a cannula can be so adjusted as to collect the whole of the bile secreted.

In cases where the apex of the gall-bladder admits of being brought well in contact with the abdominal wall, a cannula of the pattern of the one usually employed for gastric fistulæ (see p. 74) but of much smaller size, may be introduced and retained. When such is the case the free end of the cannula may be closed by an indiarubber stopper, perforated by a glass tube, to which is attached an indiarubber tube leading to a bag of the same material in which the secretion is collected.

Schiff's 'amphibolic' fistulæ. If a large fistulous aperture be established between the surface of the abdomen and the gall-bladder, without the common bile-duct being ligatured, the bile will, in its entirety, flow externally, so long as the external exit is free. If the cannula which has been fitted into the fistula is closed, either by a stopper or by an accidental plug of mucus, the bile will flow into the intestine. Such biliary fistulæ are termed by Schiff, who has employed them frequently in his researches, amphibolic fistulæ[1]. Such fistulæ offer so many advantages that it appears strange that they have not been more commonly preferred to the fistulæ with occlusion of the common bile-duct. The chief of these advantages is firstly, that the operative procedure required for their establishment is comparatively simple and that, consequently, the chances of recovery are much greater than when the bile-duct is divided. Secondly, that animals with amphibolic fistulæ do not suffer in general health to the same extent as those from whose alimentary canal the bile is continuously cut off: and thirdly, that the secretion of bile may at any time be studied under conditions which much more nearly approach the normal than can be the case if the bile is permanently withdrawn from the intestine.

Temporary biliary fistulae.

Temporary biliary fistulæ have often been established in the dog and cat. The abdomen having been opened and the common bile-duct exposed, a glass tube is introduced into it, the cystic duct being sometimes ligatured. To the glass tube is attached an indiarubber tube, by which the whole of the bile excreted is conveyed externally.

The establishment of *temporary* biliary fistulæ has often been practised in the case of the guinea-pig. The large size of the gall-bladder in this animal and the readiness with which it can be drawn to the surface obviating the principal difficulty encountered when operating on dogs. When, however, the common bile-duct of the guinea-pig is tied the animal dies in the course of about twenty-four hours[2].

SECT. 2. THE SECRETION OF BILE, THE CIRCUMSTANCES UNDER WHICH IT OCCURS, AND THE CONDITIONS WHICH INFLUENCE IT[3].

1. *Absolute amount of Bile secreted.*

The secretion of bile is continuous, though the rate at which it proceeds varies very greatly.

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[1] From ἀμφιβολία, the state of being attacked on both sides. For references to Schiff's papers and a discussion of his investigations refer to p. 278 *et seq.*

[2] The reader who desires additional information on the subject of biliary fistulæ is referred to the already quoted paper by Schwann, as well as to Kühne's *Lehrbuch*, p. 69: Heidenhain, 'Anlegung von Gallenfisteln' in Hermann's *Handbuch*, Vol. v. I. pp. 249—251: Colin, *Physiologie comparée*, Vol. I. p. 851.

[3] In the preparation of this section the Author has availed himself of much information contained in the admirable article by Professot Heidenhain, entitled 'Allgemeine Verhältnisse der Gallensecretion' in Hermann's *Handbuch*, Vol. v. I. p. 251 *et seq.*

Absolute amount of bile and bile-solids secreted by animals of various species.

From the remarks which have been made concerning the difficulties which attend the collection of the total quantity of bile secreted by animals with biliary fistulæ, the reader will be prepared for great discrepancies in the results obtained by various experimenters. When it is considered, moreover, that some observers based their estimates upon the study of the secretion in animals with temporary fistulæ (Bidder and Schmidt, Colin), whilst the majority confined their observations to animals with permanent fistulæ, one great source of discrepancy will be apparent.

The attempt has been made to express the relation between the amount of bile secreted per hour or per day in terms of the weight of the animal. Inasmuch as different individuals of the same species secrete bile differing in a remarkable degree in the amount of water it contains, most experimenters have sought to determine not only the total quantity of bile secreted but also the bile-solids.

The first result which strikes one, in considering the quantity of bile secreted, is that no comparison can be instituted in this respect between animals of different species. The smaller the animal, as a rule, the greater the quantity of bile secreted in relation to the weight of its body. It might be supposed that this stands in relation to the fact that the ratio of the weight of the liver to the total weight of the body is greater in the case of small than it is in that of large animals. As a matter of fact, however, the unit-weight of liver secretes very much more bile in the case of small than in that of large animals, as is shewn by the accompanying data (Heidenhain).

RELATION BETWEEN THE QUANTITY OF BILE SECRETED AND THE WEIGHT OF THE BODY AND LIVER. (HEIDENHAIN[1].)

	Sheep.	Rabbit.	Guinea-pig.
Ratio of Weight of Bile to Weight of Body	1 : 37·5	1 : 8·2	1 : 5·6
Ratio of Weight of Bile to Weight of Liver	1·507 : 1	4·064 : 1	4·467 : 1

Results of observations on the dog.

The dog is the animal on which by far the largest number of observations have been made, usually by the method of permanent, but occasionally by that of temporary, fistulæ.

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[1] Heidenhain, *loc. cit.*, Hermann's *Handbuch*, Vol. v. I. p. 253.

QUANTITY OF BILE AND BILE-SOLIDS SECRETED BY THE DOG (PER KGRM. OF THE BODY-WEIGHT IN 24 HOURS).

Authorities.	Fresh Bile.		Bile-solids.	
	Minimum.	Maximum.	Minimum.	Maximum.
Bidder and Schmidt	15·9	28·7	0·696	1·126
Nasse	12·2	28·4	0·400	0·784
Arnold	8·1	11·6	0·215	0·373
Kölliker and Müller	21·5	36·1	0·748	1·290
Leyden	2·9	10·4	0·19	0·58

It will be gathered from a study of the results exhibited in the above table that the quantity of bile secreted by the dog is subject to remarkable variations. In these experiments, for the most part, the quantity of food consumed by the animal has been left out of consideration.

Some relation will probably be discovered to exist between the amount of the solid matters of the bile and that of the products of the tissue waste of the organism as a whole, but the observations hitherto made have neither been directed, nor been adequate, to the elucidation of such a relation.

The secretion of bile in certain herbivorous animals contrasted with that secreted by some carnivorous animals.

In the first[1] of the subjoined tables are exhibited the average quantities of bile and bile-solids obtained in the case of the dog, cat, sheep and rabbit, by Bidder and Schmidt, and in that of the guinea-pig by Friedländer and Barisch[2].

Heidenhain has expressed in a convenient tabular form (see the second of the subjoined tables) the results of calculations based upon existing data which relate to the secretion of bile by three herbivorous animals.

THE SECRETION OF BILE AND BILE-SOLIDS IN CERTAIN HERBIVOROUS AND CARNIVOROUS ANIMALS, EXPRESSED IN TERMS OF THE WEIGHT OF BODY.

	1 kgr. of the following animals secreted in 24 hours.				
	Cat.	Dog.	Sheep.	Rabbit.	Guinea-pig.
Fresh Bile	14·50	19·99	25·41	136·84	175·84
Bile-solids	0·816	0·988	1·344	2·47	2·20

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[1] Heidenhain, Hermann's *Handbuch*, Vol. v. I. p. 252.

[2] Friedländer and Barisch, *Archiv f. Anat. u. Physiol.* 1860, p. 646.

TABLE EXHIBITING THE RELATION BETWEEN THE WEIGHT OF THE BODY AND OF THE LIVER, ON THE ONE HAND, AND THAT OF THE BILE AND BILE-SOLIDS SECRETED PER HOUR, ON THE OTHER[1].

	Sheep.	Rabbit.	Guinea-pig.
a. Mean body-weight (in grms.)	23377	1525·8	518
b. Bile secreted by 1 kgr. of Body per hour	1·109	5·070	7·320
c. Ratio of weight of liver to weight of Body	1 : 53·5	1 : 33·5	1 : 27·3
d. Bile secreted by 1 kgr. of liver per hour	62·8	169·3	185·5
e. Bile-solids secreted by 1 kgr. of liver per hour	4·13	3·74	2·67

Quantity of bile secreted by man. It is obvious that our knowledge of the amount of bile secreted by man can only be derived from observations in the very rare cases in which a biliary fistula results from some morbid process which, at the same time, hinders the flow of bile into the intestine. The fact that in the rare cases which have chanced to be observed by highly-skilled investigators and which have been elaborately investigated, the person observed has necessarily been in a more or less diseased condition detracts somewhat from their value. In three sets of observations, however, which will be referred to below (observations of Copeman and Winston, Mayo Robson, and Noël Paton) the general condition of the patient so nearly approached that of perfect health as to leave no doubt upon our mind that they furnish us with a more reliable estimate of the amount of bile secreted by the human liver than could be arrived at if we depended merely on calculations based on the widely discordant observations made on the lower animals[2].

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[1] Heidenhain in Hermann's *Handbuch*, Vol. v. I. p. 253.

[2] In addition to the observations mentioned in the text the following may be mentioned:—

1st. A case recorded by Dr George Harley (*Med. Chirurg. Transac.* 1866, p. 89) of ecchinococcus of the liver in which for a week or so during the progress of the malady bile was absent from the stools and was discharged from a fistulous opening, to the amount of between 16 and 20 ozs. per diem (quoted, at second-hand, from Noël Paton's and Balfour's paper).

2nd. A case of Dr Murchison's (*Diseases of the Liver*, 3rd ed., p. 576), in which a biliary fistula, with occlusion of the common bile duct, existed. The quantity of bile secreted by the liver in 24 hours was roughly estimated as not much under two pints (1128 c.c.), though the patient was taking very little food.

3rd. A case of Westphalen's (*Deutsche Archiv f. klin. Med.*, Vol. XI. p. 588), in which right-sided empyema had existed and in which through a thoracic fistula the whole of the bile was excreted. The bile in this case was elaborately investigated by O. Jacobson, to whose results attention will afterwards be directed. The conditions of Westphalen's case appear to the Author to have been too abnormal to permit of any conclusions as to the normal bile secretion in man being drawn from it.

Nevertheless we may be certain for reasons to be subsequently stated (p. 278 *et seq.*) that the quantity of bile obtained from a permanent biliary fistula is less than that which would be secreted under normal conditions.

Ranke's observations. In the year 1871 J. Ranke published the observations which he had made on a patient in whom, through the rupture of a multilocular hydatid cyst of the liver into the lung, bile was expectorated by the bronchi. At the time of Ranke's researches, the fistula had existed for a considerable time. At intervals, the bile appeared to be wholly expectorated, as judged from the fact that the fæces then contained no bile-colouring matter and became very rich in fat; at other times, no trace of bile was expectorated, although a catarrhal bronchial expectoration persisted, and then the fæces became normal. The catarrhal bronchial expectoration was estimated at 135 c.c. per diem. The total quantity of bile and bronchial secretion was collected and analysed on five separate occasions, the whole secretion of 24 hours being obtained. Deducting the average quantity of bronchial secretion from the total quantity of mixed fluid collected, Ranke obtained as a mean of these five sets of observations, 636 c.c. as the daily amount of bile secreted by his patient. The mean of the total solids excreted amounted to 20·62 grms. Ranke's patient secreted at the rate of 13·52 c.c. of bile and 0·44 grm. of bile-solids per kilo of his body-weight *per diem*[1].

von Wittich's case. In 1872 v. Wittich investigated the amount of bile in a woman who, as a result of cholelithiasis, had a biliary fistula. On one occasion, during a period of four hours, 88 c.c., and on another occasion during 16 hours 224 c.c. of bile were collected. The mean of these two observations gives 542·8 as the amount of bile secreted by this woman in 24 hours[2].

Observations of Yeo and E. F. Herroun. In the year 1883 Professor Yeo and Mr E. F. Herroun published an interesting set of observations in a case of biliary fistula in man. The patient (a man aet. 48) had been admitted into King's College Hospital, under the care of Dr George Johnson, F.R.S. (in a condition of extreme emaciation, weighing 112 lbs.), suffering from jaundice of six months' duration. 'On the 27th of February his tensely-filled gall-bladder was opened with aseptic precautions by Sir Joseph Lister, F.R.S., in the hope of removing the obstruction, which, however, was found to depend upon occlusion by a carcinomatous growth of the common bile-duct, and could not be made pervious .... There was no trace of bile in the fæces at any time during his stay in the hospital. The secretion was collected in sacks of black caoutchouc, which were kept aseptic by the dressings. The exclusion of atmospheric germs had the advantage of preventing cystitis and decomposition of the bile, and the attending complications[1].'

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[1] Joh. Ranke, 'Directe Bestimmung der in 24 Stunden vom ruhenden Menschen producirten Gallenmenge,' Chapter VIII. of Ranke's work, *Die Blutvertheilung und der Thätigkeitswechsel der Organe*, Leipzig, 1871.

[2] v. Wittich, 'Zur Physiologie der menschlichen Galle,' Pflüger's *Archiv*, Vol. VI. (1872), p. 181.

The patient lived two months after the operation, and on eight separate days (intervening between March 26 and April 5, 1883) (with the exception of quite insignificant losses) the whole of the bile was collected. The average quantity was found to amount to 13·2 ozs. or 374·5 c.c. in 24 hours. The average percentage of solid matters in the bile was 1·3468, so that the bile-solids eliminated per diem amounted to 5·04 grms. Both the quantity of bile secreted and the solid matters which it contained were, as it will be at once seen on comparison, decidedly less than the amounts estimated by Ranke and v. Wittich. The condition of the patient was, however, so far removed from the normal as to forbid any conclusion as to the average amount of bile secreted by healthy human subjects being drawn from them.

Observations of Copeman and Winston.

In 1889, Copeman and Winston[2] had the opportunity of investigating a case of biliary fistula in a woman æt. 26 (weighing 42·7—44·7 kilo.) under the care of Dr Bristowe in St Thomas's Hospital. In this case complete occlusion of the common bile-duct existed, which was due, as was discovered on post-mortem examination, to a calculus firmly impacted in the duct just at its junction with the intestine. On the admission of the patient, the gall-bladder had been found distended with bile. Mr Sidney Jones cut down on the gall-bladder, stitched it to the edges of the wound and laid it open, when about six or seven ounces of a glairy, semi-transparent fluid was evacuated, which was quite free from biliary colouring matter. A fistula resulted, through which the whole of the bile secreted was discharged, as evidenced by the facts that the jaundice which had existed, little by little, disappeared and the urine gave no bile reaction, whilst the stools remained of a greyish colour and contained no trace of bile. As soon as the fistula had become established a glass cannula was accurately fitted into it, and by means of an indiarubber tube the bile was led into a bottle placed on the floor beneath the bed. There was no loss of bile into the dressings and no trace of bile in either the urine or fæces, proving that the whole amount of the secretion was collected. For more than a month the total quantity secreted during the previous 24 hours was carefully measured daily at 10 p.m.; during the whole period the patient was in excellent health and her subsequent death may be looked upon as entirely accidental, having been caused by hæmorrhage, resulting from an unfortunate attempt to re-establish forcibly the continuity of the common bile-duct.

From the observations of Copeman and Winston it results that the mean secretion of bile per 24 hours was 779·6 c.c. and of bile solids 10·8 grms. The specific gravity varied between 1·0085 and 1·015[1].

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[1] 'A note on the Composition of Human Bile obtained from a Fistula.' By Gerald F. Yeo, M.D. and E. F. Herroun, *Journal of Physiology*, Vol. v. p. 116 *et seq.*

[2] Copeman, S. Monckton, and Winston, W. B. 'Observations on Human Bile obtained from a case of Biliary Fistula,' *Journal of Physiology*, Vol. x. (1889), pp. 213—231.

Observations of Mayo Robson.

In 1888 and 1889 Mr Mayo Robson[2] performed a series of very important observations on a patient in whom he had established a biliary fistula. The observations extended over a period of many months, during which the patient continuously enjoyed remarkably good health. Her weight during this period was 53 kgms. (8 stone 4½ lbs.). Many very elaborate analyses of the bile collected in this case were made by Mr Fairley. The average quantity of bile secreted, deduced from observations extending over 8 months, was 30 ozs. or very nearly 850 c.c. during 24 hours. Its specific gravity varied between 1·0085 and 1·0090, and the solid matter amounted on the average to 1·81 per cent., the total solids excreted being 15·3 grms. Mr Mayo Robson was subsequently able to establish a communication between the gall-bladder and the small intestine (by the operation of cholecystenterostomy), so that the bile resumed its normal course, the external fistulous aperture being closed. The weight of the patient subsequently increased to 60 kgms. (9 st. 6½ lbs.).

Observations of Noël Paton and J. M. Balfour[3].

The last set of observations on the amount of bile secreted by the human subject have been carried out on a patient in whom Mr John Duncan, P.R.C.S.E., had established a biliary fistula, the case being one of cholelithiasis in which the common bile-duct had become occluded. The patient was a woman aet. 51 years and weighing 70·7 kilos.

This patient differed from those investigated by Drs Copeman and Winston and by Mr Mayo Robson in so far that her condition at the time when Noël Paton and Balfour were conducting their elaborate investigations was in no sense a normal one, as proved by the abnormal variations in temperature, in the quantity of food ingested and of nitrogen excreted. The total quantity of bile excreted in 24 hours was found to amount (in the average) to 638 c.c.; the specific gravity varied between 1005·5 and 1008·2: the solids in 100 parts amounted to 1·31 and the average total weight of the bile-solids excreted in 24 hours was 8·378 grms.

In this case Dr Noël Paton[4] had the opportunity to renew his observations after an interval of one year. In the meantime the patient had returned to her home and her health had been perfectly re-established, in spite of the fact that the fistula had remained open, no drop of bile entering the intestine. The quantity of bile secreted (mean of two days' observations) was found to be 590 c.c., containing 2·3 per cent. of solids, the total bile-solids excreted during 24 hours amounting to 13·6 grms. per 24 hours.

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[1] The authors give the specific gravity as varying between 1·0085 and 1·105; in the latter figure it is obvious that a 0 in the first decimal place has been omitted.

[2] A. W. Mayo Robson, F.R.C.S., Hon. Surg. Leeds Gen. Inf., 'Observations on the Secretion of Bile in a case of Biliary Fistula,' *Proceedings of Royal Soc.* Vol. XLVII. (1890), pp. 499—524. The information as to the weight of the patient was inadvertently omitted in this paper, but has been privately communicated to the Author by Mr Mayo Robson.

[3] D. Noël Paton, M.D., F.R.C.P.Ed., and John M. Balfour, M.B., C.M. 'On the Composition, Flow, and Physiological Action of the Bile in Man.' Reprinted from Vol. III. of *Laboratory Reports* issued by the Royal College of Physicians, Edinburgh, 1891, pp. 191—240.

[4] D. Noël Paton, 'Further Observations on the Composition and Flow of the Bile in Man' (1892). Reprinted from Vol. IV. of *Laboratory Reports* issued by the Royal College of Physicians, Edinburgh.

This second set of observations (in spite of its being limited to two days) appears to the Author to possess much greater value than the first, inasmuch as the subject under investigation was in a state of robust general health. In this respect these later observations can be compared with those of Copeman and Winston and of Mayo Robson, the three sets possessing an importance which cannot be attached to any of those previously recorded.

Comparison of the results of the several observers previously referred to.

In the annexed table (p. 277) the results of the observations already referred to can be seen at a glance. Those marked with an asterisk appear to the Author to be of paramount importance, inasmuch as they relate to individuals in a satisfactory state of health. It is unfortunate that in two of the cases which were in other respects admirably investigated the weight of the patient was not ascertained with as great accuracy as the other data noted[1]. From these cases we may conclude that the amount of bile secreted by the healthy human subject (when reabsorption of bile from the intestine cannot take place) varies between a pint and a pint and a half per day, whilst the solids excreted in 24 hours amount to between 3·5 drachms to half an ounce.

Influence of Abstinence on the Flow of Bile and Variation in its Amount during the period of Digestion.

Although, as has already been stated, the flow of bile is continuous, the majority of observations point to the fact that during prolonged abstinence the secretion diminishes very greatly in quantity; the observations of Hoppe-Seyler[2] have shewn that in this condition the mineral matters of the bile are remarkably diminished, in correspondence, probably, with the greatly diminished secretion of water by the liver.

According to Kühne[3] and Hoppe-Seyler, the secretion of bile increases immediately after a meal, and the increase is maintained for about an hour. There is then a certain diminution of rate, and it is only after a considerable interval that the increased flow again sets in. The statements of various observers differ materially as to the time when this increase sets in. There is considerable evidence, however, in favour of the view that the flow of bile is particularly abundant during the 3rd and 5th hours of digestion (Voit, Kölliker

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[1] Copeman and Winston state that the weight of their patient which was at first 42·7 increased to 44·7.

Noël Paton states in his account of his second set of observations that the patient 'is fat and must weigh eleven or twelve stone.' The mean of these estimates has been taken; it is probably very near the truth, as the patient's weight a year previous was 11 st. 1½ lbs. (70·6 kilos.).

[2] Hoppe-Seyler, *Physiologische Chemie*, Berlin, 1878, p. 308.

[3] Kühne, *Lehrbuch*, p. 71.

Table exhibiting the results of different Observers on the Secretion of Bile and Bile-solids (during 24 hours) in the Human Subject.

	Ranke	V. Wittich	Westphalen	Yeo and Herroun	* Copeman and Winston	* Mayo Robson	* Noël Paton, 2nd set of observations
Sex	M.	F.	M.	M.	F.	F.	F.
Weight in kgrs.	47				42·7—44·7 Mean 43·7	53	? 73
Bile secreted in c.c.	636 c.c.	532 c.c.	498·8 c.c.	374·5 c.c.	779·6 c.c.	849·0 c.c.	590 c.c.
Bile solids in grms.	20·6 grms.		11·27 grms.	5·04 grms.	11·09 grms.	15·28 grms.	13·596 grms.
Bile secreted per kilo of body	13·52 c.c.				17·8 c.c.	16·0 c.c.	8·08
Bile-solids secreted per kilo of body	0·44 grms.				0·25 grms.	0·28 grms.	0·186

and Müller, and Hoppe-Seyler). It would also appear that a second increase in the rate of secretion occurs at a later period; here, again, the statements of observers differ greatly. The only thorough set of observations on this matter were made by Hoppe-Seyler[1] in the case of a dog with permanent biliary fistula; the bile was collected and weighed at frequent intervals (every half-hour, with occasional breaks), and the amount of its various constituents determined, by the most accurate methods, in each sample. These observations possess such unique value that the Author has exhibited the results, so far as the total quantity of bile and the amount of sodium taurocholate are concerned, in the curves shewn in Fig. 17 (p. 279).

Influence of the Nature of the Diet on the Secretion of Bile.

All that is accurately known on this subject may be summed up in a few words. It would appear that the flow of bile is *somewhat* influenced by the character of the diet, being most abundant when animals are fed on meat mixed with fat, somewhat less when fed on meat without fat, and diminishing materially if the amount of fat be excessive (Kühne). But little, if any, importance can be attached to the conclusions arrived at on this matter by Bidder and Schmidt, who fed cats on particular diets for some days before establishing temporary fistulæ and, from the amount of the bile and bile-solids secreted, ventured to formulate conclusions as to the influence of variations in diet on the biliary secretion.

Influence of the Absorption of Bile from the Intestine on the quantity of Bile secreted.

All observers who have established temporary biliary fistulæ have remarked that the quantities of bile secreted sink very materially after the operation, and Bidder and Schmidt observed that the solid constituents of the bile diminished even more than the total quantities of bile; in other words, that during the successive hours following the establishment of temporary biliary fistulæ the bile became gradually less abundant, and more watery.

In 1868, Schiff[2] pointed out that the quantity of bile secreted by dogs with biliary fistulæ diminished when the bile was withdrawn entirely from the alimentary canal, whilst it increased within 12 minutes or a quarter of an hour, when the bile was allowed again to flow into the intestine.

Schiff afterwards[3] employed two methods of investigation. In the first he established a permanent biliary fistula by the method described at page 267, the common bile duct being, as usual,

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[1] Hoppe-Seyler, *Physiologische Chemie*, p. 308.

[2] Schiff, *Giornale di scienze naturali ed economiche*, Palermo, Vol. IV. (1868), quoted by Schiff in his paper in Pflüger's *Archiv*, Vol. III. (1870). p. 598.

[3] Schiff, 'Bericht über einige Versuchsreihen angestellt in physiol. Laboratorium des Instituts zu Florenz. I. Gallenbildung, abhängig von der Aufsaugung der Gallenstoffe,' Pflüger's *Archiv*, Vol. III. (1870, pp. 598—613).

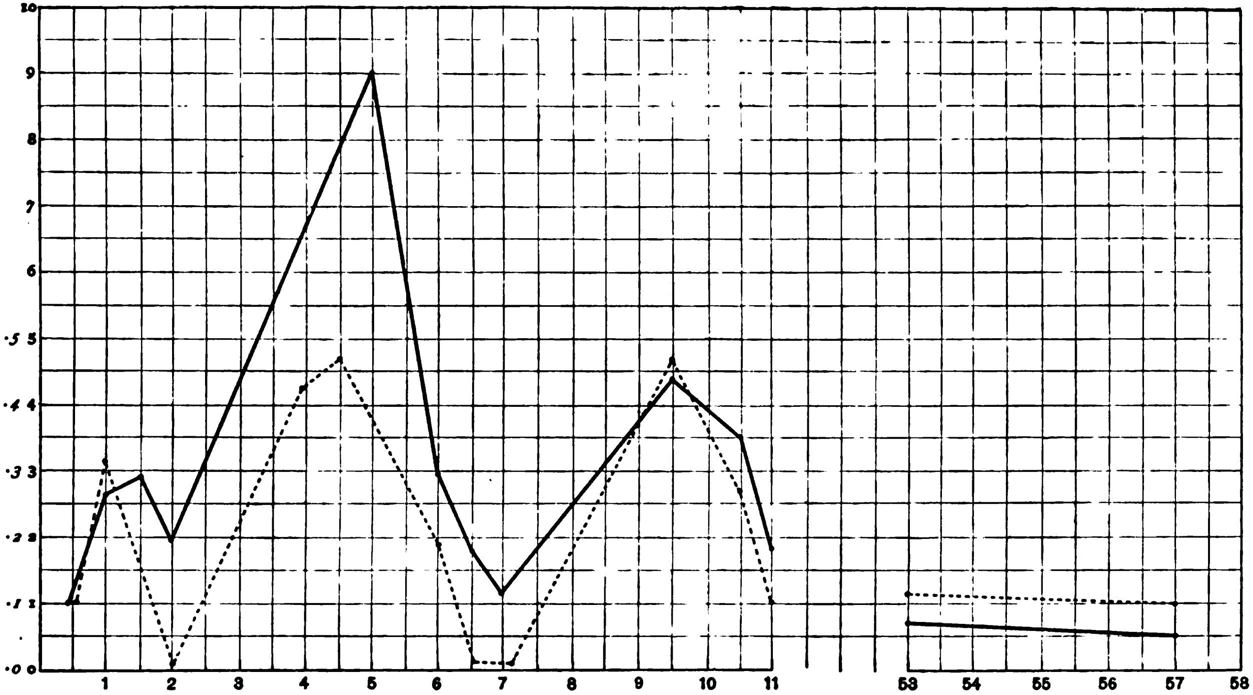

FIG. 17. VARIATIONS IN THE SECRETION OF BILE AND OF SODIUM TAUROCHOLATE DURING DIGESTION.

Curves plotted to shew the results obtained by Hoppe-Seyler in a continuous set of observations on a dog (weighing 11 kilos) with a permanent biliary fistula. The amount of bile excreted during the preceding 30 minutes was determined at each observation, as well as the proportions of its chief constituents.

The figures below the abscissa line represent hours after a meal.

The darker figures close to the ordinate line represent grammes of bile secreted. The lighter figures represent the amount of sodium taurocholate excreted in decigrammes.

The continuous curves represent the variations in the flow of bile.

The dotted curves represent the variations in the excretion of sodium taurocholate.

ligatured. In these cases, however, he established, in addition, a duodenal fistula. Having, in the first instance, observed a rapid diminution of the quantity of bile flowing from the fistula, he injected into the duodenum the bile which had been secreted by the animal during the previous hour or two hours. After 10, but more obviously after 15, minutes the quantity of bile obtained *per fistulam* increased and continued to increase during 2, 3, 5 or more successive periods of 10 minutes, according to the quantity of bile injected.

For the second series of experiments, Schiff established his so-called *amphibolic* biliary fistulæ (see page 268). As has been already stated, such fistulæ, according to Schiff, enable the experimenter, at will, to divert the whole quantity of bile from the alimentary canal, and at any time to turn it again into its proper channel. Schiff found that the quantity of bile obtained in a given period from such fistulæ was always much greater immediately after the bile had been allowed to pass normally into the intestine than when it had been flowing externally. Further, Schiff found that the introduction of solutions of bile-salts into the intestine through a duodenal fistula, caused an increase both in the total quantity of bile and of the bile-solids secreted. Upon these facts, Schiff based a theory that a circulation of bile occurs ('*Kreislauf der Galle*'): that a portion of the secretion absorbed from the intestine on reaching the liver in the portal blood furnishes the organ with a part of the material for the secretion of fresh bile. Further, finding that the increase of bile secreted followed the introduction of bile into the intestine when the portal vein had been obliterated by the method devised by Oré of Bordeaux[1], Schiff concluded that the liver was able not only to utilise, for the purposes of bile-formation, the biliary constituents which reached it in the portal blood, but also those present in the blood of the system.

The results of numerous observers substantially established the accuracy of the facts recorded by Schiff. Thus Rutherford and Vignal[2] observed that when bile was injected into the intestine the secretion of bile was increased. Tarchanoff[3] after the injection of bilirubin into the blood of a dog with biliary fistula, found that the bile pigment was increased in the bile, and his observations received full confirmation from the researches of Vossius[4], who determined the bile colouring matters by the method of spectro-photometry. Rosenkranz in a research conducted under the direction of Kunkel introduced bile into the stomach of dogs and found that the quantity secreted began to increase in the course of half-an-hour, attaining its maximum in from three to four hours and then gradually sinking[1].

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[1] Oré's method of obliterating the portal vein consists in placing a ligature, as a loop, around the vein, then closing the abdominal wound, taking care that the ends of the loop project through it. By exerting traction on these ends, phlebitis is set up which, in favourable cases, leads to occlusion of the vein.

[2] Rutherford and Vignal, *Journal of Anatomy and Physiology*, Vol. x. 1876, and xi. 1877.

[3] Tarchanoff, 'Zur Kenntniss der Gallenfarbstoffbildung,' Pflüger's *Archiv*, Vol. ix. (1874), p. 329.

[4] Vossius, 'Bestimmungen des Gallenfarbstoffes in der Galle,' *Archiv f. exp. Pathol.*, Vol. xi. (1879), pp. 426—454.

Heidenhain[2] commenting upon these results argued, with perfect justice, that, by themselves, they were not sufficient to prove that the substances conveyed to the liver in the portal blood actually passed into the secretion, for they might merely act by exciting the secreting elements of the gland to increased activity. Socoloff[3] had, indeed, endeavoured to determine whether absorbed bile acids are excreted in the bile, by injecting sodium of glykocholate into the stomach and into the blood of dogs. As the bile of the dog normally contains no salt of glykocholic acid, its appearance in the secretion would have established conclusively the truth of Schiff's hypothesis. Socoloff found, however, that under these circumstances no glykocholic acid was excreted. Admitting the accuracy of Socoloff's observations, which have however since been contradicted by Prévost and Binet[4], they do not in any respect disprove the hypothesis of Schiff, for it is conceivable, nay probable, that the glykocholic may be split up into glycocine and cholalic acid and that the latter may be utilised by the liver in the synthesis of taurocholic acid, the normal bile acid of the dog.

Wertheimer's researches.

A new and very beautiful series of researches have now placed beyond the possibility of doubt that Schiff's theory is absolutely correct in so far as the colouring matters of the bile are concerned. Taken in connection with the increase of bile and bile-solids, observed by the majority of observers, when bile is introduced into the portal system or into the general circulation, these experiments render it probable that what has been proved to be true in the case of the colouring matters likewise holds good for bile acids.

The idea which was the foundation of the conclusive experiments about to be referred to was to introduce into the blood of one animal the bile of another of different species, and of which the colouring matters are peculiar, and to observe whether the foreign bile colouring matters which have been introduced into the blood are excreted in the bile. The first experiments of this kind were made by Baldi[5], under Luciani's direction, in the Physiological Laboratory of Florence. Baldi injected ox-bile into the veins of dogs with permanent biliary fistulæ and observed, in general, an almost immediate increase in the quantity of bile secreted, the increase continuing for some hours; moreover, *the colour of the*

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[1] Rosenkranz, 'Ueber das Schicksal und die Bedeutung einiger Gallenbestandtheile,' *Verhandlungen der physiol.-med. Gesellsch. in Würzburg*, Vol. XIII. (1879), pp. 218—232.

[2] Heidenhain, 'Gallenabsonderung,' Hermann's *Handbuch*, Vol. V. I. p. 259.

[3] Socoloff, 'Ein Beitrag zur Kenntniss der Lebersecretion,' Pflüger's *Archiv*, Vol. XI. (1875), p. 166.

[4] Prévost and Binet, *Comptes Rendus*, Vol. 106 (1888), pp. 1690.

[5] D. Baldi, 'Recherches expérimentales sur la marche de la sécrétion biliaire.' Résumé de l'auteur. *Archives Italiennes de Biologie*, Vol. III. (1883), pp. 389—397.

bile obtained from the fistula assumed the green tint which is characteristic of that of the ox. Baldi's observations, taken in connection with the results of Tarchanoff and of Vossius, were sufficient to establish a presumption in favour of the view that the bile colouring matter of the ox when introduced into the blood of the dog is seized by the liver, which excretes it unaltered in the bile. But though rendering this view probable, Baldi's experiments afforded no strict scientific proof. Stadelmann[1] has shewn that when solutions containing 0·6 or 0·8 per cent. of common salt are introduced into the blood of the dog, the quantity of bile is diminished and it becomes distinctly green. The green tint noticed by Baldi might, therefore, after all, not be due to the passage into the bile of the green colouring matter of the bile of the ox.

It is to the researches of Professor Wertheimer of Lille that we owe the absolute and conclusive proof that when foreign bile colouring matters are introduced into the portal blood or into the general circulation of the dog, they are excreted unchanged by the liver.

MacMunn[2] has described as a characteristic colouring matter in the bile of the sheep and ox, a body to which he had given the name of cholohæmatin, and which is characterised by the presence in its spectrum of four absorption bands, of which one which is particularly well marked is situated between *B* and *C*, a second which is much less distinct between *C* and *D*, quite close to *D*, and two between *D* and *E*. (See Plate II. Spect. 1.)

Having in conjunction with Meyer[3] confirmed the observations of MacMunn on the spectrum of cholohæmatin, and ascertained that fresh bile of the dog, obtained from the gall-bladder, exhibits no definite absorption bands, Wertheimer[4] established temporary biliary fistulæ in dogs, and after determining that the bile flowing from the fistula exhibited no absorption bands, slowly injected varying quantities of sheep's bile into one of the femoral veins. Within a quarter of an hour from the commencement of the injection of bile into the circulation, the bile flowing from the fistula exhibited in a characteristic manner the spectrum of cholohæmatin, whilst it acquired a greenish hue. In his experiments Wertheimer observed a large increase in the flow of bile as a constant result of the injection of bile into the circulation. To his results reference will be again made in discussing the processes of absorption in the intestine.

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[1] Stadelmann, *Archiv f. experim. Pathologie*, Vol. xv. (1882), p. 337.

[2] MacMunn, 'Spectroscope in Medicine,' Lond. 1880: *Proceedings of the Royal Society*, Vol. xxxi. (1880), p. 26: *Proceedings of the Royal Society*, 1883: 'Observations on some of the Colouring Matters of Bile and Urine, with especial reference to their origin, &c.' *Journal of Physiology*, Vol. xi. (1884—85), pp. 22—39.

[3] M. E. Wertheimer et E. Meyer, 'De l'apparition de l'oxyhémoglobine dans la bile et de quelques caractères spectroscopiques normaux de ce liquide,' *Archives de Physiologie normale et pathologique*, Juillet, 1889, pp. 438—448.

[4] M. E. Wertheimer, 'Expériences montrant que le foie rejette la bile introduite dans le sang,' *Archives de Physiologie*, No. 4, Oct. 1891, pp. 724—734.

The observations of the Author, with which independent unpublished observations of Wertheimer concord, have shewn that cholohæmatin does not exist preformed in the bile of the ox or sheep at the moment of death, but that the four-banded body is rapidly formed by the oxidation of a chromogen existing in the bile when the liquid comes in contact with air. The discovery of this fact does not, however, in the least detract from the scientific value of Wertheimer's observations.

The Influence of the Blood-supply on the Secretion of Bile.

Does the liver secrete bile from materials conveyed to it by the blood of the portal vein or of the hepatic artery?

The blood-supply of the liver will be discussed in detail, in Book III., in connection with the general processes which have their seat in that organ, and the secretion of the bile will again be considered in relation to these processes. In this place it seems, however, expedient to refer to a question which has prompted many researches and given rise to endless discussion, the question, namely, as to whether the liver secretes bile at the expense of the blood conveyed to it by the portal vein or by the hepatic artery.

If the view which has been expressed at the commencement of this chapter be correct, that the bile is to be looked upon as a comparatively insignificant by-product resulting from the extremely complex and diverse chemical operations which have their seat in the liver, then the hypothesis that bile is normally formed at the expense of the blood of the hepatic artery would appear most improbable. The relations of the liver to the portal vein, and through it to the organs of digestion: the large quantity of blood which reaches the liver through the portal vein, especially during the period of high digestive activity: the exceptional manner in which the portal vein, after the manner of an artery, splits up into capillaries: besides many other anatomical and physiological considerations, afford irresistible arguments in favour of the view that the liver, which takes its origin as a mass of glandular cells in close connection with the mid-gut and which is placed in the path of the blood returning from the organs of digestion, carries on its chief operations mainly at the expense, and with the help, of the materials which the portal blood conveys to it.

Yet certain well-ascertained observations render it certain that if, through abnormal circumstances, the flow of blood through the portal vein be interrupted, the secretion of bile may continue, before the circulation of portal blood by means of collateral channels can possibly have been established. There can be no doubt that the principal part played by the hepatic artery and its branches is to supply blood to the connective tissue framework of the liver, to supply the gall-bladder and the hepatic ducts, and even to furnish the interlobular branches of the portal vein, with their *vasa vasorum*. In the liver, however, as in almost every organ, an anastomotic connection exists between the various vessels which supply it; and thus blood from the hepatic artery penetrates into the intralobular capillary network and, under abnormal circumstances, may keep up the blood-supply of the lobule so as to enable the creature to tide over the period which intervenes until the circulation through the interlobular branches of the portal vein is re-established. That the secretion of bile can go on for a time when the portal vein alone supplies blood to the liver has been shewn by the observations of a number of observers (Schiff[1], Von Asp[2], Wertheimer[3]), though an occlusion of branches of the hepatic artery rapidly leads to localised necroses of the liver substance (Cohnheim u. Litten[4]), through the cutting off of the nutritive supply to vessels, ducts, and connective tissues.

Some of the facts which prove that, when the portal vein is occluded, the secretion of bile proceeds at the expense of the blood of the hepatic artery, must now be briefly reviewed. In the first instance, cases were observed, in the human subject, in which the portal vein opened into the vena cava inferior below the liver, and yet the secretion of bile did not appear to be interfered with (Abernethy[5], Lawrence[6]). In Abernethy's case, the hepatic artery was found to be greatly dilated. In these cases, as the investigations of Kiernan[7] revealed, the lobules were supplied with venous blood through collateral channels and it cannot, therefore, be held that they offered substantial evidence in favour of the view that the hepatic artery is able, unaided, to provide the materials for the secretion of bile.

Another class of cases includes those (of which a large number have been observed in the human subject) in which, through an inflammatory process, the portal vein has been occlused by a thrombus, as well as those in which the portal vein has been experimentally occluded by the method of Oré. In the latter cases, as Schiff[8] has found, a collateral circulation, enabling portal blood to reach the interlobular portal veins, soon becomes established. For a time, however, the direct access of portal blood to the liver is cut off and yet the secretion of bile continues. The most interesting of all experiments bearing indirectly on this question are those recently performed in St Petersburgh, and to which reference will be made, at greater length, in a subsequent chapter. In these the portal vein was ligatured and, at the same time, a fistulous aperture was established between the portal vein and the inferior vena cava. About one-third of all the dogs subjected to this most difficult operation recovered from its immediate effects. A series of nervous symptoms manifested themselves which were unquestionably due to a constituent (carbamic acid) of the portal blood which is normally arrested and transformed by the liver, finding its way into the general circulation. There was no evidence, however, of an arrest of the flow of bile, during the time when the portal blood was cut off, and before a collateral circulation of portal blood to the liver was established[1].

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[1] Schiff, *op. cit.*, Pflüger's *Archiv*, Vol. III. (1870), p. 598.

[2] v. Asp, 'Zur Anatomie und Physiologie der Leber,' Ludwig's *Arbeiten*, 1873, p. 124 *et seq.*

[3] M. E. Wertheimer, 'Sur la circulation intero-hépatique de la bile,' *Archives de Physiologie*, No. 3, Juillet, 1892, pp. 577—587.

[4] Cohnheim u. Litten, Virchow's *Archiv*, Vol. LXVII. (1876), p. 153.

[5] Abernethy, *Philosophical Transactions*, 1793, p. 61.

[6] Lawrence, *Med. Chir. Transactions*, Vol. XI. (1814), p. 174.

[7] Kiernan, *Philosophical Transactions*, 1833, II. p. 758.

[8] Schiff, *Schweitz: Zeitschrift f. Heilkunde*, I. p. 1 (quoted by Heidenhain).

The conclusion which, it appears to the Author, can be legitimately deduced from the facts cited is, *that in cases of occlusion of the portal vein, the hepatic artery is able to supply the liver with sufficient blood to permit of its usual operations being performed, though in an impaired manner, and consequently the secretion of bile* (which is not a function *per se*, but rather a resultant of all the chemical processes of the organ) *is not arrested.* It must not be forgotten, however, that in these exceptional cases the hepatic artery carries to the liver blood which differs greatly from that which it usually conveys, inasmuch as the blood of the general circulation contains an unusual proportion of materials which have more or less directly reached it from the portal area.

Changes in pressure in the portal system and their influence on the flow of bile.

'The supply of blood for the liver is mainly that through the vena portæ; and this supply is not, like an arterial supply, a fairly uniform one, modified chiefly by the vaso-motor wants of the organ itself, but is dependent on what happens to be taking place in the alimentary canal and abdominal organs other than the liver itself.

'When no food is being digested and the alimentary canal is at rest, the vessels of that canal are, like those of the stomach, pancreas and salivary glands, in a state of tonic constriction; a relatively small quantity of blood passes through them; hence the flow through the vena portæ is relatively inconsiderable; and the pressure in that vessel is low. When digestion is going on all the minute arteries of the stomach, intestines, spleen and pancreas are dilated, and general exterior pressure being by some means or other maintained, a relatively large quantity of blood rushes into the vena portæ and the pressure in that vessel becomes much increased, though of course necessarily lower than the general arterial pressure. Moreover during digestion, peristaltic movements of the muscular coats of the alimentary canal are active; and these movements, serving as aids to the circulation, help to increase the portal flow. Further, the spleen is in many animals richly provided with plain muscular fibres, and in these cases seems, especially during digestion, to act as a muscular pump driving the blood onwards, with increased vigour, along the splenic veins to the liver. So that even were the liver not connected with the central nervous system by a single nervous tie, the tide of blood through the liver would ebb and flow according to the absence or presence of food in the alimentary canal[1].'

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[1] 'La Fistule d'Eck de la veine cave inférieure et de la veine porte et ses conséquences pour l'organisme. Par MM. les Drs M. Hahn, V. Massen, M. Nencki et J. Pawlow. (Travail des laboratoires de M. Nencki et de M. Pawlow.)' *Archives des Sciences Biologiques publiées par l'Institut Impérial de Médecine Expérimentale à St.-Petersbourg.* Tome I. (1892), no. 4, pp. 401—495.

That the increase in the pressure of blood in the portal vein would *ipso facto* lead to increase in the secretion of bile, corresponding doubtless to a general increase in all the activities of the liver, appears to be proved by a variety of facts. Thus when the general arterial pressure is very greatly lowered by blood-letting the flow of bile is diminished[2], though Heidenhain's own experiments shew that, as a result of hæmorrhage, the pressure in the carotid may fall to one-half its initial value without a change in the rate of the flow of bile being noticeable[3].

Section of the spinal cord in the cervical region leads to a great fall in the amount of the bile secreted, which is concomitant with the general fall of pressure throughout the vascular system. Stimulation of the spinal cord, whether direct or reflexly induced by stimulation of sensory nerves, leads to a diminished flow of bile by the contraction which it occasions in the vascular areas which receive their nervous supply through the splanchnic nerves, and of which a consequence is a diminished flow of blood through the portal vein (Lichtheim[4], Heidenhain[5]). Section of the splanchnic nerves, which is followed by a dilatation of all the arteries which furnish the blood to the radicles of the portal vein, leads to an increase in the flow of bile (Heidenhain). On the other hand, stimulation of the splanchnic nerves by causing a constriction of the arteries, and a consequent slowing of the portal stream, is followed by a diminution in the flow of bile (Munk[6]).

The stimulation of various nerves has thus an influence on the secretion of bile; this influence is, however, only an indirect one, through the variations which the stimulation occasions in the flow of blood through the liver. No evidence exists which points to the existence of any nervous apparatus, local or central, directly controlling the secretion of bile.

The Pressure under which the Bile is secreted.

In the case of the saliva, reference was made to the fact that it may be secreted though the pressure exerted by the secreted fluid is considerably higher than that of the blood circulating through the arteries which supply the gland—a fact which conclusively proves that the secretion is not an act of filtration. The secretion of the bile takes place under a pressure which, compared with that of the saliva, is very low (Friedländer u. Barisch[1]). If, however, we contrast the pressure under which the bile is secreted with the blood-pressure in the portal vein we obtain results which are in accordance with those ascertained in the case of the salivary glands.

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[1] M. Foster, *A Text-Book of Physiology*. Fifth ed. Part II. (1889), p. 436.

[2] Körner und Strube, *Studien des physiologischen Instituts zu Breslau*, II. (1863), p. 101.

[3] Heidenhain, 'Gallenabsonderung,' Hermann's *Handbuch*, Vol. v. I. p. 263.

[4] Lichtheim, 'Ueber den Einfluss der Rückenmarksreizung auf die Gallenabsonderung,' *Inaug. Diss.* Berl. 1867 (quoted by Heidenhain).

[5] Heidenhain, *Studien d. physiol. Inst. zu Breslau* IV. (1868), p. 226.

[6] J. Munk, 'Ueber den Einfluss sensibler Reizung auf die Gallenausscheidung,' Pflüger's *Archiv*, Vol. VIII. (1874), p. 151.

Heidenhain[2], measuring simultaneously the pressure under which bile is secreted and the pressure in a branch of the inferior mesenteric vein of dogs, found that the bile-pressure invariably exceeded the portal blood-pressure by a considerable amount. In the following table are stated his results, the pressure being expressed in terms of a column of solution of sodium carbonate.

TABLE EXHIBITING THE RELATION BETWEEN THE PRESSURE UNDER WHICH THE BILE WAS SECRETED AND THE PRESSURE IN THE SUPERIOR MESENTERIC VEIN IN FIVE EXPERIMENTS ON THE DOG. (HEIDENHAIN.)

	Bile-pressure.	Blood-pressure (superior mesenteric vein).
1.	220 mm. sol. of Sod. Carbonate	90 mm. sol. of Sod. Carbonate
2.	175 " " "	67 " " "
3.	204 " " "	90 " " "
4.	110 " " "	50 " " "
5.	180 " " "	65 " " "

Taking the arithmetic mean of the ratios of bile-pressure : blood-pressure in these five experiments it appears that the former was nearly twice and a half as great as the latter (2·45 : 1).

Re-absorption of secreted bile.

If the outflow of bile be prevented as by the application of a ligature to the common bile-duct, or through its occlusion by a gall stone or a plug of mucus, the bile already secreted is rapidly re-absorbed. The biliary constituents *ultimately* pass into the blood and are excreted by the urine. If their amount be sufficiently large, however, the colouring matter soon stains the conjunctiva and later the skin, the condition of *ictorus* or jaundice being established.

Researches conducted, under Ludwig's direction, by Fleischl[3] led to the interesting discovery that the bile is not re-absorbed by the blood-vessels of the liver, but by the lymphatics of the organ, whence it makes its way into the thoracic duct which carries it into the blood. This fact was proved by applying a ligature to the common bile-duct of dogs, in whose thoracic duct a cannula was inserted permitting the collection externally of the whole of the lymph. Whilst this fluid contained large quantities of the biliary constituents, the blood-serum was entirely free from them.

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[1] v. Friedländer und C. Barisch, 'Zur Kenntniss der Gallenabsonderung' (aus d. phys. Institut zu Breslau), *Archiv f. Anat. u. Phys.* 1860, see p. 659 *et seq.*

[2] Heidenhain, original experiments published in his article 'Gallenabsonderung,' Hermann's *Handbuch*, Vol. v. I. p. 269.

[3] Fleischl, 'Von der Lymphe und den Lymphgefässen der Leber,' Ludwig's *Arbeiten*, 1874, p. 24.

Small quantities of undecomposed bile acids are normally present in the chyle of the thoracic duct. Tappeiner was able to isolate and identify them in 150 c.c. of chyle obtained from the thoracic duct of a dog in full digestion[1].

SECT. 3. THE PHYSICAL CHARACTERS AND THE REACTION OF THE BILE.

The bile flowing from the hepatic ducts is a non-viscous liquid, the product of the activities of the liver cells. In the gall-bladder, and perhaps even in the cystic and common bile-ducts, it becomes mixed, however, with the product of the secretion of the glands situated in the mucous membrane, and acquires a viscidity, formerly supposed to be due to mucin, but which is caused by a peculiar body now classed among the 'nucleo-albumins'; we shall designate this body the '*mucoid nucleo-albumin*' of the bile.

Colour. The perfectly fresh and normal bile of man and of carnivorous animals generally (dog, cat, pig) is of a golden red colour. When examined spectroscopically the liquid cuts off more or less of the violet and blue ends of the spectrum; it either exhibits no definite absorption bands or at most on suitable dilution, a shadowy absorption band near F, occupying approximately the same position as that exhibited by urobilin.

In the very interesting case of biliary fistula in a woman recorded by Dr Copeman and Mr Winston[2], the bile presented from first to last 'a deep olive tint in which green certainly preponderated over the yellow.' These observations have led Copeman and Winston to surmise that the normal colour of human bile may be olive-green, and that the colour usually described as characteristic of the bile of man may be due to a reducing process occurring during the sojourn of the bile in the gall-bladder. In Jacobsen's description of the bile secreted in Westphalen's case the bile is described as a '*grünlich braungelbe* Flüssigkeit.'

The fresh bile of herbivorous animals is of a pure grass-green or olive-green colour (rabbit, sheep, goose) or it is of a colour in which green predominates, but in which a red tint is perceptible (ox, sometimes sheep). It absorbs the violet and some of the blue rays of the spectrum, but before it has come in contact with air presents no definite absorption bands; in other words, at the time of death the bile does not contain any body possessing the optical properties of the cholohæmatin of MacMunn, but a chromogen from which cholohæmatin is rapidly developed (Gamgee). The main colouring matter on which the green colour depends exhibits no absorption bands.

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[1] H. Tappeiner, 'Ueber die Aufsaugung der gallensauren Alkalien im Dünndarme,' *Sitzungsber. d. Wiener Academie d. Wissensch.*, Vol. 77 (1878), Abth. III.

[2] S. Monckton Copeman, M.A., M.D., and W. B. Winston, 'Observations on Human Bile obtained from a case of Biliary Fistula,' *Journal of Physiology*, Vol. X. (1889), 213—231, see p. 218 *et seq.*

The brown tint which human bile, collected in the gall-bladder *post mortem*, usually possesses, and which frequently is exhibited by the bile obtained from biliary fistulæ, when, through septic invasion, catarrh of the gall-bladder has been set up, is due to a change which the normal pigment has undergone.

When exposed to the air, bile which when fresh had a reddish-yellow colour, may assume a grass-green tint similar to that of the herbivora. Conversely, the green bile of the ox when decomposing in the absence of air, loses its green colour and becomes brown. The causes of these changes in colour will be treated of in discussing the colouring matters of the bile.

Taste. The bile possesses at first a characteristic sweet-bitter, almost immediately followed by a purely bitter, taste, which is imparted to it by the bile acids which it contains.

Odour. The fresh bile is usually destitute of smell. The bile of the ox and sheep when concentrated on the water bath often exhales a faint musk-like odour.

Density. The density of the bile which is flowing freely from a fistula is much lower than that of bile obtained from the gall-bladders of animals *post mortem*.

According to Frerichs, the specific gravity of human (gall-bladder) bile varies between 1·03 and 1·04. According to v. Gorup-Besanez, the specific gravity of human bile varies between 1·0105 and 1·032. On the other hand, the most reliable determinations of the density of bile from biliary fistulæ in man give results which never approached the higher limit assigned by Gorup-Besanez. O. Jacobsen[1], in his examination of the bile of a man with biliary fistula, found the density to vary between 1·0105 and 1·0107 (t. 17·5° C.). Copeman and Winston[2] found it to range between 1·0085 and 1·0105. Yeo and Herroun[3] found the density to vary between 1·008 and 1·0082. In Mayo Robson's case, Fairley found it to vary between 1·0085 and 1·0090. Noël Paton and Balfour observed variations between 1·0054 and 1·008.

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[1] Osc. Jacobsen, 'Untersuchung menschlicher Galle.' *Ber. d. deutsch. Chem. Gesellschaft.* Vol. VI. (1873), p. 1026.

[2] Copeman and Winston, *loc. cit.*, p. 221.

[3] Yeo and Herroun, 'A note on the composition of Human Bile obtained from a fistula.' *Journal of Physiology*, Vol. V. (1883—84), p. 118.

Reaction of the bile. The bile is a feebly alkaline liquid. According to Bidder and Schmidt its alkalinity depends on admixture with the secretion of the gall-bladder. In order to determine its reaction the bile should be previously diluted.

SECT. 4. ENUMERATION OF THE CONSTITUENTS OF THE BILE. THE CONSTITUENTS WHICH ARE SPECIFIC OF THE SECRETION.

The bile is an aqueous solution containing as its principal and specific constituents (1) the sodium salts of certain conjugated acids comprised under the general designation of *bile acids*; (2) certain colouring matters, included under the general term of *bile colouring matters*, of which the principal are *bilirubin*, the characteristic colouring matter of the bile of the carnivora, and *biliverdin*, which is characteristic of the bile of the herbivora. Amongst the characteristic constituents of the mixed bile should perhaps be also included (3) the mucoid nucleo-albumin which is mixed with the bile in the gall-bladder. We speak of the bile acids and bile colouring matters being specific constituents of the bile and specific products of the liver, *inasmuch as these substances are not found in the blood or in the tissues of animals from which the liver has been removed*[1]. We cannot with equal positiveness speak of the mucoid nucleo-albumin being a specific constituent, as bodies of somewhat similar composition and reactions occur elsewhere; besides, this constituent is not a product of the activity of the hepatic cells and must be looked upon, in a sense, as an adventitious constituent of the bile.

In addition to the bodies which are the specific products of the activity of the hepatic cells and the mucoid nucleo-albumin, the bile contains cholesterin, neutral fats, soaps, lecithin, or its products of decomposition, traces of a diastatic ferment, mineral matters, amongst which a small quantity of iron is invariably found, and gases of which the chief is CO_2.

SECT. 5. GLYKOCHOLIC ACID, TAUROCHOLIC ACID, HYOGLYKOCHOLIC ACID, HYOTAUROCHOLIC ACID, CHENOTAUROCHOLIC ACID.

Introductory Observations.

If the bile of the ox be mixed with animal charcoal and evaporated to dryness on the water bath, a pulverisable residue is obtained. This when treated with absolute alcohol yields a colourless or faintly yellow solution, containing no trace of the bile colouring matters, but which holds in solution the sodium salts of the bile acids. If now anhydrous ether be added to this alcoholic solution, a white turbidity is at first produced and, as the quantity of ether added is increased, a white amorphous precipitate falls to the bottom and adheres, in part, to the walls of the vessel in which the precipitation occurs. If the process of drying of the bile has been complete and the alcohol and ether subsequently employed anhydrous, the snow-white precipitate in the course of some hours or some days exhibits tufts of beautiful needles[1]. To this crystalline precipitate the name of Plattner's crystallised bile is given. It is composed of the sodium salts of glykocholic and of taurocholic acids.

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[1] In old blood extravasations, microscopic crystals of so-called hæmatoidin occur which appear to be identical in crystalline form, colour, spectroscopic characters and chemical reactions with bilirubin.

The bile of all animals contains either a glykocholic or a taurocholic acid or a mixture of these acids. Glykocholic acid, on the one hand, is a nitrogenous acid which, under the influence of hydrolytic agencies, splits up into amido-acetic acid (glycocoll) and into a non-nitrogenous acid, termed cholalic acid. Taurocholic acid, on the other hand, contains not only nitrogen but sulphur and, under hydrolytic agencies, splits up into amido-ethylsulphonic acid (taurine) and into cholalic acid. The cholalic acid of the bile of certain animals (as the pig and the goose) has a slightly different composition from that of the normal cholalic acid, and thus we may have a glykocholic or a taurocholic acid not absolutely identical with those of the ox. These observations will render intelligible the statement previously made that the bile of all animals contains a glykocholic or a taurocholic acid or a mixture of the two.

As will be afterwards stated in detail, the bile of herbivora contains glykocholic acid as its principal bile acid, whilst the bile of carnivora contains taurocholic acid as its chief or sole bile acid. The bile of man contains a preponderance of glykocholic acid, taurocholic acid being sometimes absent.

The bile of nearly all classes of animals, with the exception of that of fishes, contains sodium salts of the bile acids. In salt water fishes, however, the bile acids are in combination with potassium; in fresh water fishes both potassium and sodium salts exist, the latter predominating. The same curious fact has been observed in the bile of certain '*Chelonii.*'

The Early History of researches on the Bile Acids.

The views of Fourcroy and Vauquelin.

When we consider how important a part the older physicians ascribed to the bile in the causation of disease we cannot be surprised to find that this secretion was amongst the first to be subjected to investigation. Passing over, for the present, the observations made before chemistry had attained to the position of a positive science, the first to be noticed are those of Fourcroy and Vauquelin[2], whose views do not appear to have differed materially from those which had prevailed concerning the nature of the bile during the latter half of the 18th century, viz. that it was a secretion of soap-like character. This view was based on the alkaline nature of the bile, on the resinous precipitate obtained on the addition of acids to it, and on the fact that a part of the acids added remained in solution in combination with sodium.

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[1] Plattner, *Ann. d. Chem. u. Pharm.*, Vol. LI. (1844).

[2] Fourcroy, 'Système des connaissances chemiques.' Quoted by Berzelius in his article 'Galle' in Wagner's *Handwörterbuch der Physiologie*, Vol. I. (1842), p. 516. The Author has not been able to see the original.

The researches of Thenard[1]. It is in the almost simultaneous observations of Thenard and of Berzelius published in the first years of the present century that we trace the germs of our knowledge of the bile acids. Thenard experimented upon the bile of the ox. He discovered the important fact that neutral and basic lead acetates when added to bile cause a precipitate; this he decomposed by means of dilute nitric acid and obtained a coloured resin-like body which he designated 'résine de la bile.' This body must have consisted of a mixture of glykocholic and choloidic acids with colouring matters. The filtrate from the first precipitate, when treated with a large excess of lead acetate, yielded a second yellow precipitate, from which, by the action of H_2S, he obtained a soluble extractive matter to which, because of its bitter and sweet taste, he gave the name of picromel (from πικρός, pungent, bitter; and μελί, honey).

The researches of Berzelius. Shortly after Thenard's researches, Berzelius shewed that when acetic acid is added to bile it precipitates a body which had until then been considered to be albumin, but which he declared to consist of mucin secreted by the gall-bladder, but does not precipitate any resin-like body from bile. On adding, however, sulphuric or hydrochloric acid to bile, he obtained a precipitate consisting of a resin-like body. On decomposing this precipitate with barium carbonate or with lead carbonate (according to the acid which he had employed in the precipitation) he obtained a watery solution of a substance possessing the taste of bile, and which, he assumed, formed compounds with acids which were insoluble in water in the presence of an excess of acid. To this supposed proximate principle of the bile, Berzelius applied the name of biliary substance ('*Diese Substanz nannte ich Gallenstoff*')[2]. As we now know, the biliary substance of Berzelius must have consisted of impure glykocholic acid.

The discoveries of Leopold Gmelin and of Redtenbacher. In the year 1826 in the celebrated work which he wrote in association with Tiedemann, Leopold Gmelin[3] announced the results of his researches on the bile. He declared that the biliary substance of Berzelius, no less than the 'résine de la bile' and the picromel of Thenard, consisted of mixtures of bodies. From the biliary resin he succeeded in separating, however, a crystalline acid to which he gave the name of cholic acid, and which was the acid to which Lehmann afterwards applied the name by which we now know it, viz. glykocholic acid. Amongst a large number of hypothetical proximate principles, the discovery of which depended upon errors of method or of interpretation, Gmelin placed a crystalline body which he had separated from Thenard's biliary resin; this crystalline body, which he believed to exist in a free condition in the bile, and which he designated biliary asparagine ('*Gallenasparagin*'), is the body we now know as taurine. Its composition was unknown to Gmelin, who was not even aware that it contained the element sulphur. It is a remarkable fact that both Demarcay and Dumas who subsequently made ultimate analyses of taurine, having failed to test it for sulphur, obtained results which led to the formula $C_2H_7NO_5$[1, 2]. It was only in 1846 that Redtenbacher[3] discovered the presence of sulphur in taurine, and shewed that the empirical formula $C_2H_7NO_5$ must be replaced by $C_2H_7NSO_3$.

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[1] Thenard, 'Mémoire sur la Bile,' lu à l'Institut le 2 floréal, an 13. *Mémoires de Physique et de Chimie de la Société d'Arcueil.* Tome I. Paris, 1807, p. 23—45.

[2] Berzelius, *Fördäsningar i Djurkemien,* II. p. 248, Stockholm, p. 1808. Quoted by Berzelius in his article 'Galle' in Wagner's *Handwörterbuch.*

[3] Tiedemann und Gmelin, *Die Verdauung nach Versuchen,* Vol. I. (1826), p. 63 *et seq.*

Demarcay's investigations.

The next enquirer whose researches we are called upon to notice was Demarcay. His work formed the basis of an independent report to the French Academy by Dumas and Pelouze[2], the former of whom submitted to ultimate analysis some of Demarcay's products. This observer concluded that the bile contains the sodium salt of a single acid to which he gave the name of choleic acid (acide choléique), which was analysed by Dumas as well as by himself and to which the empirical formula $C_{42}H_{72}N_2O_{12}$ was assigned. When boiled with acids he believed that this acid split up into an acid which he termed choloidic acid, $C_{38}H_{60}O_7$, and into taurine, $C_4H_{14}N_2O_{10}$ $(C = 6)$. When boiled with caustic alkalies choleic acid was, according to Demarcay, split up into ammonia and cholic acid, $C_{42}H_{72}O_{10}$. Demarcay's choleic acid was, in reality, taurocholic acid in which he failed to find the sulphur, as he likewise did in taurine. In spite of his mistakes in these particulars, Demarcay was the first to recognise the conjugate nature of a bile acid and to shew that taurocholic acid splits up, when heated with acids, into a nitrogenous body, and into a non-nitrogenous acid.

The researches of Adolf Strecker.

It is to the long continued and masterly researches of Adolf Strecker that we owe the greater part of our knowledge of the bile acids and of their compounds, and the description of these bodies which will follow is based in great part upon his work. His first

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[1] H. Demarcay, 'Ueber die Natur der Galle.' *Annalen der Pharmacie*, Vol. XXVII. (1838), pp. 270—291.

[2] Pelouze und Dumas, 'Bericht über vorstehende Abhandlung an die Akademie in Paris (Auszug).' *Annalen der Pharmacie*, Vol. XXVII. (1838), pp. 292—295: also in *Comptes Rendus*, 1838, no. 8, 2^me séance.

[3] Jos. Redtenbacher, 'Ueber die Zusammensetzung des Taurins.' *Annalen der Chem. u. Pharm.*, Vol. LVII. (1846), pp. 170—177.

research on the bile was published in association with Gundlach in the year 1847 and concerned the bile of the pig, in which they discovered the sodium salt of an acid which they named hyocholic acid and which we now term hyoglykocholic acid[1]. In 1848 Strecker published in two parts his great research on the bile of the ox[2,3]. Starting from the cholic acid of Gmelin, Strecker succeeded, by boiling this body with a solution of barium hydrate, in decomposing it, obtaining as products of decomposition glycocoll and a non-nitrogenous acid, obviously the same as that which Demarcay had named cholic acid. Strecker called this acid cholalic acid, the name by which it is now generally known, and assigned to it the formula $C_{24}H_{40}O_5$.

Passing over his researches on the products of decomposition of cholalic acid, we have to bring into special relief the second great result of his research on ox bile. Having separated the glykocholic acid which exists in that bile by precipitating it with neutral lead acetate, he treated the filtrate with basic lead acetate, and obtained a second precipitate containing the lead salt of an acid in whose composition not only N but S entered; to this acid which (after Lehmann) we now call taurocholic acid, Strecker applied the term choleic acid (choleinsäure).

Although unable to obtain taurocholic acid in a crystalline condition, he succeeded, by the same method which he had employed in the case of glykocholic acid, in decomposing it into taurine (which Redtenbacher had already shewn to contain sulphur and to which he had ascribed a correct empirical formula), and into cholalic acid. Strecker had thus succeeded in shewing that the two bile acids, like hippuric acid, are conjugate acids which readily split up into a non-nitrogenous acid common to the two bile acids, and into amido-compounds—the N-containing glycocine on the one hand, and the N- and S-containing taurine on the other.

In a third great paper[4] Strecker recorded his researches on the bile of various animals, investigating, *inter alia*, the mineral constituents of the bile of fishes, and completing the research which he had commenced with Gundlach on hyoglykocholic acid.

Glykocholic acid, $C_{26}H_{43}NO_6$.

Glykocholic acid is the principal bile acid in the bile of man, the ox and other herbivorous animals, and occurs in combination with sodium. It is not found in the bile of carnivorous animals.

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[1] Dr C. Gundlach und Dr Ad. Strecker, 'Untersuchung der Schweinegalle.' *Annalen d. Chem. u. Pharm.*, Vol. LXII. (1847), pp. 205—232.

[2] Ad. Strecker, 'Untersuchung der Ochsengalle.' Erste Abhandlung. *Ann. d. Chem. u. Pharm.*, Vol. LXV. (1848), pp. 1—37.

[3] Ad. Strecker, 'Untersuchung der Ochsengalle.' Zweite Abhandlung. *Annalen d. Chem. u. Pharm.*, Vol. LXVII. (1848), pp. 1—60.

[4] Ad. Strecker, 'Beobachtungen über die Galle verschiedener Thiere.' *Annal. d. Chem. u. Pharm.*, Vol. LXX. (1849), pp. 149—197.

Modes of preparation.

1. *From Plattner's 'crystallised bile'* (see p. 291). The crystalline precipitate obtained by adding ether to a solution of dried decolourised bile in absolute alcohol is dissolved in water and dilute sulphuric acid is added to the solution, until a permanent and dense turbidity is produced. The liquid is set aside when, after some hours, glykocholic acid separates in the form of fine silky needles. It is collected on a filter, washed with distilled water, pressed between filter paper and then dissolved in alcohol, only as much of this liquid being employed as suffices to dissolve the acid. To the alcoholic solution, many times its volume of ether is added, when pure glykocholic acid separates out, in the form of long silky needles.

2. *By precipitation with lead acetate.* Decolourised extract of ox bile is dissolved in water and the solution is treated with a solution of lead acetate which produces a precipitate composed almost entirely of glykocholate of lead. This precipitate is collected, washed, drained, and is mixed with alcohol. A solution containing an excess of sodium carbonate is then added and the mixture evaporated to dryness. In this process a double decomposition occurs, which results in the formation of lead carbonate and sodium glykocholate. From the dry residue which, in addition to these salts, contains an excess of sodium carbonate, the sodium glykocholate is dissolved out by means of absolute alcohol. The alcoholic solution is then evaporated to dryness, the residue dissolved in water, decolourised by heating with pure animal charcoal, filtered and treated with dilute sulphuric acid, which causes the separation of glykocholic acid. This process is very instructive for the student, but does not so readily, or so uniformly, yield a pure product as the one first described.

3. *By Hüfner's method*[1]. Perfectly fresh ox bile is treated with a few drops of strong hydrochloric acid which causes the separation of the mucoid nucleo-albumin. The liquid is then filtered and to each 100 c.c. of the filtrate 5 c.c. of concentrated hydrochloric acid are added. The liquid being placed in a stoppered cylinder, 30 c.c. of ether are added for every 100 c.c. of bile, the whole shaken and placed in the cold. In the most favourable cases, the separation of crystals of glykocholic acid commences at once; usually, however, some hours elapse before the separation occurs, when the mixture is found to have been converted into an almost solid magma of crystals. The ether having been diluted, the crystals are washed in ice-cold water until the washings are colourless; they are then dissolved in the smallest possible quantity of boiling water which, on cooling, deposits them in a colourless and pure condition.

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[1] G. Hüfner, 'Schnelle Darstellung von Glykocholsäure. *Journ. f. prakt. Chemie*, Vol. x. (1874), p. 267.

This method which is the simplest, and when successful the best, of all which are available, fails very frequently. Thus Marshall[1] found that in only 22·2 per cent. of all the trials which he made with the fresh bile of oxen slaughtered in Philadelphia did the separation of glykocholic acid occur. It would appear that when ox bile contains a considerable quantity of taurocholic acid, in addition to glykocholic acid, the former prevents the precipitation of the latter by hydrochloric acid, even in the presence of ether.

Physical and chemical properties.

Glykocholic acid usually crystallises in the form of colourless transparent needles. By concentrating its alcoholic solution at a boiling temperature, it is obtained in the form of thin four-sided prisms. It requires 303 parts of cold and 102 parts of boiling water to dissolve it. It is readily soluble in alcohol, but almost insoluble in ether. It is soluble in solution of the alkaline hydrates and their carbonates, and displaces the carbonic acid from the latter.

Solutions of glykocholic acid in alcohol or water possess a combined bitter and sweet taste and an acid reaction. Glykocholic acid is readily dissolved, without undergoing decomposition, by concentrated sulphuric, hydrochloric, and acetic acids, at ordinary temperatures. It is soluble in glycerin. The glykocholates of the alkalies and alkaline earths are readily soluble in water and in alcohol, whilst the glykocholates of the heavy metals are either insoluble or only sparingly soluble in water. A watery solution of an alkaline glykocholate is precipitated by neutral lead acetate, the precipitate is soluble in hot alcohol and, on cooling, separates out partly in a powdery condition and partly in flakes. Solutions of alkaline glykocholates are able to dissolve small quantities of saponifiable soaps, of lecithin, and of cholesterin.

Free glykocholic acid and its salts are dextrogyrous.

Specific rotation (α)D of glykocholic acid dissolved in alcohol = + 29°·0.

Sodium glykocholate $C_{26}H_{42}NaNO_6$

May be prepared from pure glykocholic acid by dissolving it in a solution of sodium carbonate, evaporating to dryness, dissolving the residue in absolute alcohol and precipitating by the addition of a large excess of anhydrous ether. The sodium salt soon separates out, as was described in the case of crystallised bile. It is very soluble in water; 100 parts of alcohol at 15°C. dissolve 3·9 parts of the salt. When heated, it melts and takes fire, and ultimately leaves an easily fusible mass, containing much sodium cyanate.

The specific rotation (α)D of an alcoholic solution = + 25°·7.

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[1] John Marshall, 'Ueber die Hüfner'sche Reaktion bei amerikanischer Ochsengalle.' *Zeitschrift f. physiol. Chemie*, Vol. xi. (1887), p. 233.

Products of decomposition of glykocholic acid.

When subjected to long continued boiling with diluted hydrochloric or sulphuric acid, or when heated for 12 hours in a sealed tube with a concentrated solution of barium hydrate in a water bath, glykocholic acid combines with a molecule of water and splits up into glycocoll and cholalic acid, thus:

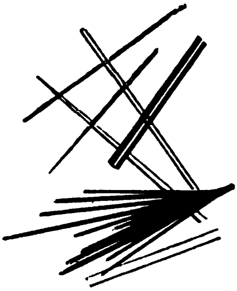

FIG. 18. SODIUM GLYKOCHOLATE.

$$\underbrace{C_{26}H_{43}NO_6}_{\text{Glykocholic acid}} + H_2O = \underbrace{C_{24}H_{40}O_5}_{\text{Cholalic acid}} + \underbrace{CH_2(NH_2)COOH}_{\text{Glycocoll}}$$

Cholonic acid. $C_{26}H_{41}NO_5$

According to Hoppe-Seyler[1], when a solution of glykocholic acid in concentrated sulphuric acid is warmed, an amorphous precipitate separates consisting of cholonic acid (cholonsäure); this acid is insoluble in water, but readily soluble in alcohol. Cholonic acid has not been obtained in a crystallised condition. It is dextrogyrous and its specific rotation is approximately the same as that of glykocholic acid. It is distinguished both from glykocholic and cholalic acids by the insolubility of its barium salt in water. Cholonic acid, it will be observed, differs from glykocholic acid in containing one molecule less of H_2O.

Pettenkofer's reaction.

Glykocholic acid, as indeed cholalic acid and all its immediate derivatives, exhibit the reaction known by the name of its discoverer[2] 'Pettenkofer's reaction.' In its original form the test is performed as follows: the solution of the substance to be tested is (after the separation of albuminous substances) placed in a test tube or in a porcelain capsule and to it are added 2 or 3 drops of a 10 per cent. solution of cane sugar. When the contents of the tube have been shaken, strong and pure sulphuric acid is added drop by drop, care being taken that the temperature of the mixture does not rise above 70°C., lest the cane sugar be carbonised and the dark colour thus caused should conceal the reaction. If bile acids are present, the fluid first of all becomes opalescent, then the opalescence clears, and the liquid becomes successively of a pale cherry-red, then of a dark carmine-red, and lastly of a beautiful purple-violet tint. The reaction does not occur instantly and the tube should be set aside for some minutes and then examined before a negative conclusion is drawn from the experiment. In order to avoid the troublesome carbonising of cane sugar which is very apt to occur, Drechsel suggested a very useful modification of Pettenkofer's test, which consisted in substituting phosphoric acid for sulphuric acid[1]. To the liquid to be tested sugar is added and, instead of sulphuric acid, phosphoric acid made by diluting the syrupy acid with one-sixth of its volume of water; the test tube containing the mixture is then placed in boiling water, when the characteristic reaction developes.

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[1] *Handbuch der physiologisch- und pathologisch-chemischen Analyse.* Sechste Auflage neu bearbeitet von F. Hoppe-Seyler und H. Thierfelder. Berlin, 1893. See p. 211.

[2] Pettenkofer, 'Notiz über eine neue Reaction auf Galle und Zucker.' *Annal. d. Chemie u. Pharmac.*, Vol. LII. (1844), p. 90—96.

A very important modification of Pettenkofer's reaction we owe to Mylius[2], who found that the reaction depended on the production of furfurol which is generated by the action of sulphuric acid on the sugar employed, and suggested that a solution of furfurol in water, of 1 per mille, should be substituted for sugar. According to v. Udranszky[3] to 1 c.c. of the alcoholic solution suspected to contain a bile acid is added a single drop of 1 p.m. furfurol solution, and then 1 c.c. of concentrated sulphuric acid, the mixture being if necessary cooled. By this method a perceptible reaction is obtained with quantities of cholalic acid not exceeding $\frac{1}{20}$th—$\frac{1}{30}$th of a milligramme.

It has long been known that many substances, some of them constituents of the animal body, give reactions with cane sugar or with cane sugar and sulphuric acid or with sulphuric acid alone, which are exceedingly similar to the reaction which is observed with the bile acids. Albumin, acids of the fatty series, amyl-alcohol, morphia, and phenol compounds which occur in the urine, all give reactions with sugar and sulphuric acid, which resemble Pettenkofer's reaction so closely that were it not for the method of spectroscopic observation we should be unable to pronounce an opinion concerning the identity or non-identity of the colouring matters which are produced in each case.

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[1] E. Drechsel, 'Eine Modification der Pettenkofer'schen Reaction auf Gallensäuren.' *Journ. f. prakt. Chemie*, Vol. xxiv. (1881), p. 44. 'Anwendung von Phosphorsäure statt Schwefelsäure bei der Pettenkofer'schen Reaction auf Gallensäuren.' *Journ. f. prakt. Chem.*, Vol. xxvii. (1883), p. 424.

[2] F. Mylius, 'Zur Kenntniss der Pettenkofer'schen Gallensäure-reaction.' *Zeitschrift für physiol. Chemie*, Vol. xi. (1887), p. 492.

[3] v. Udranszky, 'Ueber Furfurolreaktionen.' *Zeitschrift f. physiol. Chemie*, Vol. xii. (1888), p. 355—395, and Vol. xiii. (1889), p. 248.

The spectrum of Pettenkofer's reaction. [1,2,3]

When a solution in which Pettenkofer's reaction has been developed is examined spectroscopically, if the concentration be great the whole of the violet and blue rays are absorbed and a marked absorption band is seen occupying the interspace between *b* and *E* and extending beyond *E* towards *D*; the centre of this band is about λ 527; a much fainter shade may be seen on the refrangible side of *D*, the centre of which corresponds approximately to λ 585. If, by the addition of alcohol, we dilute the liquid, or if we examine a thinner stratum, as the absorption of the more refrangible end of the spectrum diminishes, a band on the violet side of *F* is seen, the centre of which corresponds approximately to λ 487. It is the two absorption bands, near *E* and near *F* which characterise the secretion. See Plate II. (Spectrum 6).

The products of decomposition and the means of determining glykocholic acid in the bile will be considered in the sequel.

Hyoglykocholic acid, $C_{27}H_{43}NO_5$.

This acid is characteristic of the bile of the pig, in which it exists in combination with sodium and was first investigated by Gundlach and Strecker[4] and afterwards by Strecker[5]; by these observers it was designated hyocholic acid[6].

Method of separation.

Decolourised pig's bile is saturated with crystallised sodium sulphate, which precipitates the sodium compound of hyoglykocholic acid. The precipitate is washed with saturated solution of sodium sulphate, dissolved in alcohol, precipitated with ether, and the ethereal precipitate, after solution in water, is treated with hydrochloric acid, which causes the separation of the bile acid. The process may be simplified by directly dissolving to sodium sulphate precipitate in water and adding hydrochloric acid to the solution.

Properties.

Hyoglykocholic acid has not been hitherto obtained in the crystalline form; it is a white resinous body insoluble in water, slightly soluble in ether, but readily soluble in absolute alcohol,

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[1] Koschlakoff und J. Bogomoloff, 'Unterschied zwischen der Pettenkofer'schen Gallensäure- und Eiweiss-reaction' (aus d. klin. Laborat. d. Herrn Prof. Botkin in St Petersburgh). *Centralblatt d. med. Wissenschaft*, 1868, p. 529—531. This paper gives an accurate description of the spectrum of Pettenkofer's reaction. A second paper by Bogomoloff, 'Ueber die Spectraleigenschaften der Gmelin'schen Reaction der Galle, der Gallensäure Chromogene, und der Pettenkofer'sche Probe' (*Centralblatt*, 1869, p. 529), is full of erroneous statements concerning the spectrum of Pettenkofer's reaction.

[2] J. L. Schenk, 'Die modificirte Pettenkofer'sche Gallenprobe.' *Anat. physiol. Untersuchungen* von J. L. Schenk. Wien, Braumüller, 1873. Abstracted in Maly's *Jahresbericht*, Vol. II. (1874), p. 232.

[3] C. A. MacMunn, 'Studies in Medical Spectroscopy.' *Dub. Journ. Med. Science*, 1877, and *The Spectroscope in Medicine*, London, 1880, p. 165.

[4] Gundlach und Strecker, 'Untersuchung der Schweinegalle.' Liebig's *Annalen*, Vol. LXII. (1847), p. 205—232.

[5] Strecker, 'Beobachtungen über die Galle verschiedener Thiere.' *Annalen*, Vol. LXX. (1849), p. 149—197.

[6] From ὗς, pig, and χολή, bile.

the alcoholic solution possessing a bitter taste. The alkaline salts are soluble in water; the salts which it forms with the alkaline earths and the heavy metals are insoluble in water but, for the most part, are soluble in alcohol. The alkaline hyoglykocholates are almost completely precipitated when their solutions are saturated with NaCl, NH_4Cl and by alkaline sulphates.

When boiled with alkalies or dilute sulphuric acid, hyoglykocholic acid splits up into glycocoll and hyocholalic acid $C_{25}H_{40}O_4$.

According to Jolin[1], pig's bile contains, in addition to Strecker's acid, a second one, also amorphous, to which the name of β-hyoglykocholic acid has been assigned.

Taurocholic acid, $C_{26}H_{45}NSO_7$.

This acid occurs, though in smaller quantities than glykocholic acid, in the bile of the ox, the sheep and other herbivorous animals. It is the sole bile acid present in the bile of the dog; in that of man it is occasionally absent (Jacobsen), and, if present, its amount is subject to great fluctuations. It occurs in the bile in combination with sodium.

Modes of preparation.

1. *By Plattner's process from the bile of the dog.* Strecker found that when the bile of the dog is subjected to the process, which, when applied to the bile of the ox yields the so-called crystallised bile, it likewise furnishes a crystalline precipitate which, he surmised, consisted of an alkaline taurocholate.

Hoppe-Seyler[2] not only confirmed the observation but, by decomposing the salt precipitated by ether from the alcoholic extract of dog's bile, obtained taurine and cholalic acid and further proved that the specific rotation of the taurocholic acid of the dog agreed with that of the acid in ox bile. J. Parke[3], working under Hoppe-Seyler's direction in Tübingen, repeated these observations. The crystalline ethereal precipitate of dog's bile was dissolved in water, precipitated with lead acetate and a little ammonia; the precipitate was well washed, then boiled with absolute alcohol, the alcoholic solution filtered hot, the filtrate treated with H_2S as long as a precipitate of PbS separated, again filtered, the filtrate concentrated at a moderate temperature and then precipitated by a great excess of ether. The syrupy precipitate consisting of free taurocholic acid, after some time, became converted in great part into needle-shaped crystals possessing a silky lustre; even after the prolonged action of ether, a portion of the precipitate refused to crystallise; on the addition, however, of a drop or two of alcohol, the whole crystallised.

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[1] S. Jolin, 'Ueber die Säuren der Schweinegalle.' *Zeitschrift f. physiol. Chem.*, Vol. XI. (1887), p. 417: Vol. XII. (1888), p. 512, and Vol. XIII. (1889), p. 205.

[2] Hoppe-Seyler, *Journ. f. prakt. Chemie*, Vol. LXXXIII. (1868), p. 83.

[3] J. Parke, 'Ueber die Taurocholsäure.' *Med. Chemisch. Untersuchungen aus dem Lab. für angewandte Chemie zu Tübingen*, herausgegeben von Dr F. O. Hoppe-Seyler. Erstes Heft. Berlin, 1866. See pp. 160 and 161.

2. *From ox bile by precipitation with basic lead acetate.* Having by means of neutral lead acetate precipitated the whole glykocholic acid (see page 295) a further addition of solution of lead acetate and of ammonia causes the precipitation of lead taurocholate.

This precipitate is treated exactly as was directed in the case of the corresponding salt of glykocholic acid. The acid obtained in this way is, however, amorphous.

Physical and chemical properties.

Taurocholic acid can, as has already been stated, be obtained in a crystalline condition, though with much greater difficulty than glykocholic acid. The needle-shaped crystals rapidly deliquesce in air; they are readily soluble in water and alcohol, the solutions having a strongly acid reaction. On evaporating the aqueous solution to dryness it undergoes decomposition. Solutions of taurocholic acid possess a bitter-sweet taste. Both aqueous and alcoholic solutions are dextrogyrous, the rotatory power of the latter being greater than that of the former.

Sp. Rot. $(a)D$ of taurocholic acid in alcoh. sol. $= +25^{\circ}\cdot 0$[1].

The alkaline salts of taurocholic acid are neutral, possess a taste which is first of all sweet and then bitter, are hygroscopic, readily soluble in water and alcohol. Their aqueous solutions foam like solutions of soap. Solutions in absolute alcohol are precipitated by a large excess of ether. The solutions of the alkaline salts are more stable than the free acid, so that they can be evaporated to dryness without undergoing decomposition. When acetic or any mineral acid, is added to the solution of a pure taurocholate, neither turbidity nor precipitation follows. Solutions of alkaline taurocholate dissolve and emulsify small quantities of neutral fats; they possess the property, which is probably of great physiological importance, of holding small quantities of cholesterin in solution.

Aqueous solution of taurocholic acid, or solutions of the alkaline salts of taurocholic acid if acidified with dilute hydrochloric acid completely precipitate solutions of albumin, of acid albumin and of parapeptone (antialbumat). Solutions of albumoses and of peptones, on the other hand, throw down the acid itself in the form of a milky precipitate[2].

Potassium taurocholate $C_{26}H_{44}KNSO_7$ is in many fishes the only taurocholate present (Strecker).

Sodium taurocholate $C_{26}H_{44}NaNSO_7$, of which the properties have already been described, has in alcoholic solution a specific rotation $(a)D = +24\cdot 5$ (Hoppe-Seyler).

Products of decomposition.

Taurocholic acid and its salts are much more unstable than glykocholic acid and its salts. When heated with alkalies or with acids, it yields, therefore, much more readily the primary products of decomposition or derivatives of these.

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[1] Parke, *op. cit.* p. 161.

[2] R. Maly u. F. Emich, 'Ueber das Verhalten der Gallensäuren zu Eiweiss und Peptonen, &c.' *Monat. f. Chemie*, Vol. 4, 1883, pp. 89—120.

Boiling with saturated baryta water or still better heating in a sealed tube with the same reagent, at the temperature of boiling water, causes taurocholic acid to split up into taurine and cholalic acid, thus:—

$$\underbrace{C_{26}H_{45}NSO_7}_{\text{Taurocholic acid.}} + H_2O = \underbrace{C_{24}H_{40}O_5}_{\text{Cholalic acid.}} + \underbrace{C_2H_7NSO_3}_{\text{Taurine.}}$$

When exposed to the air, as well as in the alimentary canal, under the influence of putrefactive organisms, a similar decomposition occurs.

Recognition of taurocholic acid.

Like glykocholic acid, taurocholic acid exhibits Pettenkofer's reaction.

In a mixture of bile acids both the presence and the amount of taurocholic acid are determined by fusing with a mixture of sodium carbonate and saltpetre and determining the presence and the amount of the sulphuric formed.

Hyotaurocholic acid, $C_{27}H_{45}NSO_6$.

This acid, which was formerly known as hyocholeic acid (Strecker), is the sulphur-containing acid of pig's bile. It has not hitherto been obtained in a pure condition. When boiled with alkalies or dilute acids it yields as products of decomposition taurine and hyocholalic acid, $C_{25}H_{40}O_4$ (see p. 307).

Chenotaurocholic acid, $C_{26}H_{49}NSO_6$ (?).

This acid, which is present in the bile of the goose[1], was first investigated by Heintz and Wislicenus[2] and then by Otto[3]. It exists in the bile as a sodium salt. It is separated by the method which, with ox bile, yields Plattner's crystallised bile; the ether precipitate is however, in the case of the bile of the goose, at first, amorphous. It is washed with a concentrated solution of sodium sulphate, dried, dissolved in absolute alcohol, and the clear filtered solution is precipitated by means of ether (which contains water). In the course of time small rhombic tables of sodium chenotaurocholate separate, which when dried at 140° C. have the composition $C_{29}H_{48}NaNSO_6$.

The salt is dissolved in water, the solution precipitated with basic lead acetate, the precipitate suspended in alcohol, decomposed by means of H_2S and filtered. The filtrate on evaporation leaves the acid as an amorphous residue, soluble both in water and alcohol.

When boiled with barium hydrate, chenotaurocholic acid yields taurine and chenocholalic acid, $C_{27}H_{44}O_4$.

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[1] Hence the name (χήν, ὁ and ἡ, gander, goose).

[2] Heintz u. Wislicenus, *Pogg. Ann.*, Vol. CVIII. p. 547.

[3] Otto, *Zeitsch. f. Chemie*, 1868, p. 633.

SECT. 6. THE ACIDS RESULTING FROM THE DECOMPOSITION OF THE CONJUGATED BILE ACIDS.

1. *Cholalic Acid*, $C_{24}H_{40}O_5$. *Syn.: Cholic Acid.*

This acid is not a constituent of fresh bile; it is found, however, in decomposed bile, and, in minute quantities, in the contents of the small and large intestine and in the faeces. As has already been said, it is chiefly known as the product of the decomposition of the normal bile acids by alkalies or acids.

Modes of preparation. In the preparation of cholalic acid, alkalies are preferable to acids, inasmuch as with the former the process of decomposition of the bile acids is better under control. When acids are employed, cholonic acid, an anhydride of glykocholic acid, is apt to be formed (see p. 297), and at a later stage dyslysin, an anhydride of cholalic acid.

1. *By the Action of Caustic Baryta on ox bile*[1]. 500 c.c. of bile are treated with 75 grms. of barium hydrate and the mixture boiled for 24 hours, on a sand bath, in a flask furnished with an inverted condenser (see Fig. 20, p. 309). The liquid is allowed to cool and filtered. Concentrated hydrochloric acid is then added, which decomposes the barium cholalate and throws down impure cholalic acid. The precipitate is thoroughly kneaded in water, and is dissolved in solution of caustic soda; it is then mixed with 30 grms. of pure animal charcoal with which it should remain in contact for some days; the liquid is filtered, again precipitated with hydrochloric acid, thoroughly washed with water and dissolved in the smallest possible quantity of hot alcohol. The alcoholic solution is treated with water until turbidity appears. On the liquid being cooled, cholalic acid separates in the form of hard transparent tetrahedra and in clumps of radiating needles.

2. *By Boiling Ox Bile with Caustic Soda*[2]. Ox bile is mixed with one-fifth its weight of a 30 per cent. solution of sodium hydrate and is boiled for 24 hours, as in process 1. The liquid is then saturated with CO_2 and evaporated almost to dryness; the residue is extracted with strong alcohol, which dissolves the sodium salts of cholalic acid as well as those of choleic and stearic acids. The solution is then diluted with water until the quantity of alcohol in it does not exceed 20 per cent., and a dilute solution of barium chloride is added so long as a precipitate occurs, which is then separated by filtration. The filtrate should yield no further precipitate with barium chloride; to it is then added hydrochloric acid which precipitates the cholalic acid. The fluid with the precipitate

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[1] F. Mylius, 'Notiz über die Darstellung und Zusammensetzung der Cholsäure.' *Zeitschrift f. physiol. Chem.*, Vol. XII. p. 262.

[2] Mylius, *op. cit.*

is set aside, when it becomes crystalline; it is purified by being recrystallised from its solution in ethyl alcohol, and finally from its solution in methyl alcohol.

Physical and chemical properties.

Cholalic acid may be obtained in the crystalline form, either anhydrous or with one molecule of water of crystallisation.

From its solutions in ethyl alcohol it separates in the form of colourless, transparent tetrahedra or octahedra, having the composition $C_{24}H_{40}O_5 + C_2H_6O$. Analogous crystalline compounds with methyl and allyl alcohols respectively are obtained when it is crystallised from solutions in these bodies. It also forms compounds with the volatile oils of mustard.

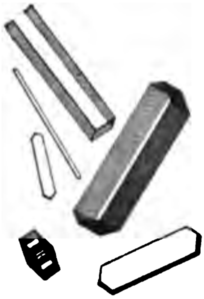

FIG. 19. CHOLALIC ACID.

Cholalic acid is very sparingly soluble in cold water requiring 4000 times its weight for solution. It is soluble in 750 parts of boiling water; the acid which separates from the solution on cooling is anhydrous and occurs in microscopic crystals. From solutions in dilute acetic acid, cholalic acid separates in rhombic plates containing one molecule of water of crystallisation. The water of crystallisation is expelled by long continued heating at 100°—120° C. The water-free acid melts at 195° C. Cholalic acid is very sparingly soluble in ether; 1000 parts of 70 per cent. alcohol dissolve 48 parts of the acid. It is somewhat soluble in glycerin and in almond oil.

Solutions of cholalic acid possess the bitter-sweet taste which characterises the conjugated bile acids. Cholalic acid in the free condition, as well as in combination with bases, is dextrogyrous.

Cholalic acid forms crystalline salts with the alkaline metals, which are readily soluble in water but less so in alcohol. From a watery solution which is not too dilute they are precipitated in a crystalline form by the addition of ether. The sodium salt, $C_{24}H_{39}NaO_5$, has the specific rotation $(\alpha)D = +31^{\circ}\cdot 4$. The barium salt $(C_{24}H_{39}O_5)_2Ba$ crystallises in silky needles, and is soluble in 30 parts of cold and 23 parts of boiling water; it is still more soluble in alcohol. The ready solubility of barium cholalate is taken advantage of in separating it from the higher members of the fatty acid series. The lead and silver salts are insoluble in water but soluble in hot alcohol.

Mylius's iodine compound.

Cholalic acid forms with iodine a remarkable compound, which furnishes us with a reaction by which we can distinguish it from the conjugated bile acids, as well as from other acids closely connected with cholalic acids, as choleic acid and hyocholalic acid.

0·02 grm. of crystallised cholalic acid dissolved in 0·5 grm. of alcohol is treated with one-tenth of a c.c. of decinormal solution of iodine, and water is then very gradually added to the solution. The liquid, which was at first brown, becomes pervaded by a magma of microscopic needles which by reflected light exhibit a metallic lustre, and by transmitted light appear of a blue colour. This compound, like the compound of starch with iodine, is dissociated with remarkable facility by heat, and the addition of water suffices to break it up into iodine and cholalic acid.

Empirical formula of cholalic acid.

Considerable difference of opinion has existed as to the empirical formula of cholalic acid. Latschinoff[1] maintained that the formula of the acid should be $C_{25}H_{42}O_5$ instead of $C_{24}H_{40}O_5$, the formula based on Strecker's researches. Mylius[2], however, from his own very elaborate and conclusive researches, as well as from the analyses of cholalic acid made by Strecker and by Schotten[3] conclusively establishes that the empirical formula $C_{24}H_{40}O_5$ agrees much better with experimental facts than the formula proposed by Latschinoff.

Action of certain oxidising agents on cholalic acid.

1. *Dehydrocholalic acid*, $C_{24}H_{40}O_5$. By the action of a 10 per cent. solution of chromic acid on a 10—15 p.c. solution of cholalic acid in glacial acetic acid, at ordinary temperatures, Hammarsten[4] obtained the acid to which he assigned the name dehydrocholalic acid; this acid crystallises from alcohol in the form of needles. It does not give Pettenkofer's reaction. Its alkaline salts dissolved in alcohol are precipitated by the addition of ether and furnish a crystallisation resembling Plattner's crystallised bile.

The sodium salt in aqueous solution has a specific rotation

$$(a)D = +27°·64.$$

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[1] P. Latschinoff, 'Ueber die Gallensäuren.' *Ber. d. d. chem. Gesellsch.*, Vol. XX. (1887), 1043—1053; 'Ueber die empirische Formel der Cholsäure.' *Ibid.* Vol. XX. p. 3274.

[2] F. Mylius, 'Ueber die Cholsäure.' *Ibid.* Vol. XX. 1968 *et seq.*

[3] C. Schotten, 'Zur Kenntniss der Gallensäuren.' *Zeitschrift f. physiol. Chemie*, Vol. X. (1886), pp. 175—217. Refer to 'II. Taurocholsäure,' pp. 190 and 191.

[4] O. Hammarsten, 'Ueber Dehydrocholalsäure ein neues Oxydationsproduct der Cholalsäure.' *Nova Acta Reg. Soc. Scient. Upsal.*, Serie III. 1881. A full abstract of the paper by its author appeared in Maly's *Jahresbericht*, Vol. XI. (1882), pp. 313—315.

2. *Bilic Acid*, $C_{24}H_{34}O_8$. By the action of potassium dichromate and sulphuric acid on cholalic acid Clève[1] obtained bilic acid —an acid which is devoid of bitter taste, which does not exhibit Pettenkofer's reaction, which is sparingly soluble in water but readily soluble in alcohol, and separates from its alcoholic solutions in crystals belonging to the rhombic system. Its specific rotation $(a)j = +47^{\circ}{\cdot}4$.

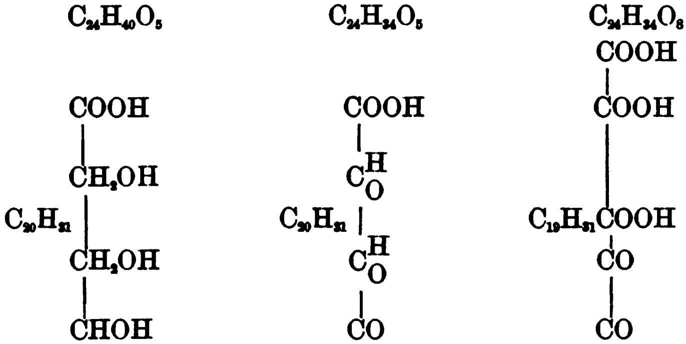
Structural formula of cholalic acid and its relation to dehydrocholalic acid and bilic acid.

According to Mylius cholalic acid may be represented by the formula,

$$C_{20}H_{31}\left\{\begin{array}{l} CHOH \\ (CH_2OH)_2 \\ COOH. \end{array}\right.$$

This formula does not attempt to explain the structure of the nucleus of cholacic acid, but to indicate that cholalic acid is a monobasic acid in which are two primary alcohol groups and one secondary alcohol group. The hypothetical relation according to Mylius[2] between cholalic acid and its derivatives is exhibited in the three subjoined structural formulae (Mylius, slightly modified by Neumeister[3]):

Cholalic acid. Dehydrocholalic acid. Bilic acid.



$$\begin{array}{ccc}
C_{24}H_{40}O_5 & C_{24}H_{34}O_5 & C_{24}H_{34}O_8 \\
\begin{array}{rl} & COOH \\ & | \\ & CH_2OH \\ C_{20}H_{31} & | \\ & CH_2OH \\ & | \\ & CHOH \end{array}
&
\begin{array}{rl} & COOH \\ & | \\ & C^{H}_{O} \\ C_{20}H_{31} & | \\ & C^{H}_{O} \\ & | \\ & CO \end{array}
&
\begin{array}{l} COOH \\ | \\ COOH \\ | \\ C_{19}H_{31}COOH \\ | \\ CO \\ | \\ CO \end{array}
\end{array}$$

Desoxycholalic acid, a product of the reduction of cholalic acid.

From bile which had been decomposed by the action of putrefactive bacteria, Mylius[4] separated, in addition to cholalic acid, an acid with the composition $C_{24}H_{40}O_4$, to which he assigned the name desoxycholalic acid, indicating that it differs from cholalic acid in containing less oxygen. He also obtained this acid by causing sodium cholalate to digest for some days at about 40° C. with decomposing pancreas.

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[1] P. T. Clève, 'Sur les produits d'oxydation de l'acide cholalique.' *Bull. Soc. Chimique*, Vol. xxxv. 373—379, 429—438. Abstracted in Maly's *Jahresbericht*, Vol. xi. (1882), p. 316. The Author has not seen the original.

[2] Mylius, 'Ueber die Cholsäure.' *Ber. d. d. chem. Gesellsch.* Vol. xx. p. 1968 *et seq.*

[3] Neumeister, *Lehrbuch der physiologischen Chemie*, Erster Theil, 1893, see p. 162.

[4] Mylius, 'Ueber die Cholsäure.'

This acid possesses a pure bitter taste, devoid of the sweet taste which is characteristic of cholalic acid and the conjugative bile acids. It is much more readily soluble in cold alcohol than cholalic acid. On spontaneous evaporation of the alcoholic solution a syrupy residue is obtained which slowly crystallises. The acid melts at temperatures between 160° and 170°. Its alkaline salts are readily soluble in water but possess a character which distinguishes them from the alkaline cholalates, viz. they are precipitated from their solutions in an oily form by the addition of 10 per cent. solutions of sodium hydrate. The barium salt of this acid dissolved in very weak solutions of ammonia is precipitated in the cold by barium chloride.

Dyslysin[1] $C_{24}H_{36}O_3$.

Anhydrides of Cholalic Acid. When bile, or one of the bile acids (preferably cholalic acid), is subjected to long continued boiling with hydrochloric acid, or when cholalic acid is heated to 300° C. (Strecker[2]), or according to Hoppe-Seyler[3] only to 200° C., a dark, resinous body is obtained, which is insoluble in water, alcohol, dilute acids and alkalies, but which is sparingly soluble in ether and soluble in aqueous solutions of cholalic acid and its alkaline salts. This body which received its name from Berzelius, who first obtained it in an impure condition, and was first investigated by Strecker, is dyslysin and has the composition $C_{24}H_{36}O_3$. The body is obviously an anhydride of cholalic acid, from which it differs by containing two molecules less of water, and into which it can be reconverted by boiling for an hour with an alcoholic solution of potassium hydrate, when it passes into solution; this reconversion also occurs on fusion with potassium hydrate. From the potassium cholalate, thus regenerated, pure cholalic acid can be obtained.

Choloidic Acid $C_{24}H_{38}O_4$ *? ?* By this name has been described and analysed by several chemists[4] a substance obtained by the action of acids on cholalic acid, of which the composition nearly agrees with the formula $C_{24}H_{38}O_4$, presumably an anhydride of cholalic acid, differing from it by containing one molecule less of water, whilst, as we have seen, dyslysin contains two molecules less. Hoppe-Seyler[4] has shewn that it is impossible, by the means which were employed to separate it, to obtain a pure substance, and he has shewn that choloidic acid is a mixture of dyslysin and cholalic acid, and that the choloidates are cholalates mixed with dyslysin. Probably, however, as Hoppe-Seyler points out, such an anhydride as choloidic acid ($C_{24}H_{38}O_4$) actually exists, though no proof of the fact has hitherto been furnished.

Hyocholalic Acid, $C_{25}H_{40}O_4$.

Is obtained by decomposing hyoglycocholic acid by boiling it with barium hydrate. It has been obtained in the form of warty, crystalline masses, which are readily soluble in alcohol and ether, but not in water. It exhibits Pettenkofer's reaction. When boiled with hydrochloric acid it yields an anhydride, hyodyslysin $C_{25}H_{38}O_3$.

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[1] From δυς and λύσις; so called because of its insolubility in water and alcohol.

[2] Strecker, *Annalen*, Vol. LXVII. (1848), p. 1 *et seq.*

[3] Hoppe-Seyler quoted by Maly, *Hermann's Handbuch*, Vol. v. II. p. 139.

[4] Hoppe-Seyler, *Physiologische Chemie*, p. 291.

Chenocholalic Acid, $C_{27}H_{44}O_4$.

Is obtained by decomposing taurochenocholic acid by boiling with barium hydrate. The acid occurs usually in a resinous form, but has been obtained in crystals by adding water to the alcoholic solution, the crystallisation taking place after a long period.

Chenocholic acid is insoluble in water, but readily soluble in alcohol and ether. Its barium salt $(C_{27}H_{43}O_4)_2Ba$ is insoluble in water. Chenocholalic acid exhibits Pettenkofer's reaction[1].

Fellic Acid ? ?

According to Schotten[2], there is obtained by the decomposition of the bile acids of man, in addition to cholalic acid, an acid to which he assigns the formula $C_{23}H_{40}O_4$.

Choleic Acid, $C_{25}H_{42}O_4$? ?

According to Latschinoff[3], when the bile acids of the ox are decomposed, in addition to normal cholalic acid, there is obtained an acid to which he has assigned the above name and formula, and the barium salt of which is insoluble. Further and independent researches are needed in order to prove whether this acid is not a secondary product and whether the above formula correctly represents its composition.

SECT. 7. THE AMIDO-ACIDS WHICH RESULT FROM THE DECOMPOSITION OF THE CONJUGATED BILE ACIDS: GLYCOCINE AND TAURINE.

Glycocine[4] $(C_2H_5NO_2)$.

(*Amido-acetic acid* $CH_2(NH_2)COOH$.)

Occurrence. Glycocine has only been found uncombined (0·4—0·7 per cent.) in the muscular tissue of *Pecten irradians* (Chittenden)[5]. It is obtained as a primary product of the decomposition, by acids and alkalies, of glykocholic acid, of hyoglycocholic acid and of hippuric acid. It is also one of the products of decomposition obtained when gelatine is subjected to long boiling with barium hydrate, or with dilute sulphuric acid, as well as when it undergoes digestion by trypsin. It is also yielded when fibroin (one of the constituents of silk) is subjected to Schützenberger's process, and when spongin is boiled with dilute sulphuric acid.

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[1] Consult on Chenocholalic acid, Heintz u. Wislicenus, *Poggend. Annalen*, Vol. CVIII. p. 547. Otto, *Zeitschrift f. Chemie*, 1868, p. 635.

[2] Schotten, 'Zur Kenntniss der Gallensäuren.' *Zeitschrift f. physiol. Chemie*, Vol. X. (1886), p. 175, and 'Ueber die Säuren der menschlichen Galle.' *Ibid.* Vol. XI. (1887), p. 268.

[3] P. Latschinoff, 'Ueber eine der Cholsäure analoge neue Säure.' *Ber. d. d. chem. Gesellsch.* Vol. XVIII. (1885), p. 3039: Vol. XIX. (1886), p. 1140: Vol. XX. (1887), p. 1043.

[4] Originally glycocoll (γλυκύς, sweet, and κόλλα, glue), from the fact of its being a sweet body obtained by the decomposition of gelatine (or glue).

[5] Chittenden, "Contributions from the Sheffield Laboratory of Yale College," No. 35. From the *American Journal of Science and Art*, Vol. X. July 1875. The Author has not seen the original paper, of which a long abstract appeared in Maly's *Jahresbericht*, Vol. V. p. 204 *et seq.*

Modes of preparation.

1. *From glykocholic acid.* Pure glykocholic acid is added to a saturated solution of barium hydrate and the mixture boiled in a flask with an inverted condenser for eight hours (see fig. 20). The liquid having been allowed to cool, sulphuric acid is added to it; this precipitates both cholalic acid and barium. The filtrate containing an excess of sulphuric acid is heated with pure barium carbonate, and then filtered. The filtrate is concentrated and set aside to crystallize.

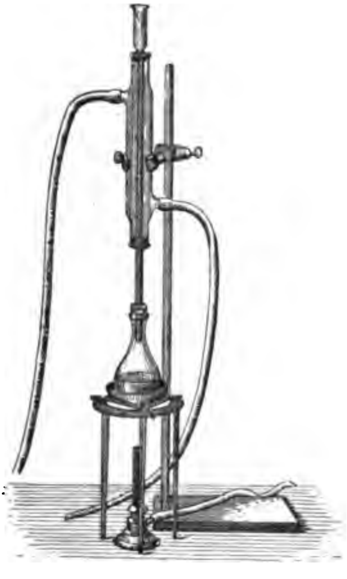

FIG. 20. FLASK, PLACED ON SAND BATH, AND FITTED WITH AN INVERTED CONDENSER.

2. *From hippuric acid.* Glycocine is most economically as well as most readily obtained by decomposing hippuric acid (benzoyl-amido-acetic acid), by boiling it with hydrochloric or sulphuric acids. The reaction which occurs is represented by the following equation:

$$\underbrace{C_6H_5.CO.NH.CH_2.COOH}_{\text{Hippuric acid.}} + H_2O = \underbrace{C_6H_5.COOH}_{\text{Benzoic acid.}} + \underbrace{CH_2.NH_2.COOH}_{\text{Glycocine.}}$$

The decomposition should be effected in the apparatus shewn in fig. 20. One part of hippuric acid is boiled in it for 10—12 hours with four times its weight of dilute sulphuric acid (1 of acid to 6 of water, by weight). At the end of this time, the contents of the flask are poured, *with caution*[1], into a capsule, allowed to settle for 24 hours, and then filtered. The benzoic acid, which has separated out, is washed with cold water, the filtrate is concentrated by evaporation, and shaken with ether, so as to free it from all traces of benzoic acid; the acid solution is then largely diluted and exactly neutralised by means of barium hydrate (or by heating with barium carbonate). The precipitate is allowed to settle, washed by decantation with boiling water, the filtrate concentrated by evaporation; in the event of an excess of barium hydrate having been added, CO_2 is passed through it, the liquid boiled and then filtered and concentrated until crystals commence to form on the surface. The liquid is then set aside to crystallise during 24 hours; the mother liquid is separated from the crystals and again concentrated, the process being repeated so long as crystals continue to separate. The glycocine thus obtained is purified by being recrystallised from water[2].

Synthesis of glycocine.

Glycocine may be synthetically obtained by various methods. The most interesting is perhaps by the action of ammonia on bromacetic acid or on chloracetic acid, the reactions being shewn in the following equations:—

(1) $CH_2.Br.COOH + NH_3 = CH_2.NH_2.COOH + HB$,

(2) $CH_2.Cl.COOH + 2NH_3 = CH_2.NH_2.COOH + NH_4Cl$.

Physical and chemical properties.

Glycocine occurs in fine, hard, colourless, crystals, unaltered by exposure to air, and which present the form either of rhombohedra or of four-sided prisms.

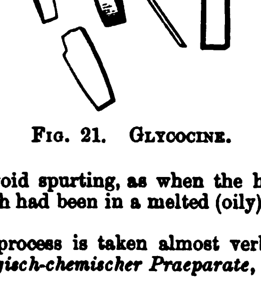

FIG. 21. GLYCOCINE.

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[1] Caution is needed to avoid spurting, as when the hot liquid is poured into the capsule, the benzoic acid which had been in a melted (oily) condition suddenly solidifies, and violent ebullition ensues.

[2] The description of this process is taken almost verbatim from Drechsel's *Anleitung zur Darstellung physiologisch-chemischer Praeparate*, p. 9.

Glycocine possesses a sweet taste; it is soluble in 4·3 parts of water, very slightly soluble in spirits of wine and insoluble in cold absolute alcohol and ether: it is very sparingly soluble in boiling alcohol. Its solutions possess an acid reaction. When heated to 232°—236° C. glycocine becomes brown, evolves gas bubbles and melts. A solution of glycocine is coloured red by ferric chloride.

Compounds of glycocine. Glycocine forms compounds with acids, with bases, and with neutral salts.

1. When a solution of glycocine is treated with an excess of of HCl and evaporated on the water bath, it yields crystals of the hydrochlorate having the composition CH_2NH_2COOH, HCl, which are readily soluble in water and in alcohol.

2. Boiling solutions of glycocine dissolve freshly precipitated cupric hydrate, and deposit on cooling dark blue needles, which are insoluble in alcohol, and soluble with difficulty in cold water, and which have the composition represented by the formula $(CH_2(NH_2)COO)_2Cu + H_2O$. Similarly, by substituting silver oxide for cupric hydrate, there is formed the compound having the composition $CH_2(NH_2)COOAg$.

3. Glycocine forms crystalline compounds with chlorides, nitrates and sulphates. The following are examples:—$CH_2(NH_2)COOH, AgNO_3$: $2(CH_2(NH_2)COOH), BaCl_2$.

Methods of identification. Having separated glycocine, it is recognised by its crystalline form, its great solubility in water, its insolubility in alcohol and ether, and the solubility of its crystalline hydrochlorate in alcohol. Further, if the quantity of the body be sufficient, its taste, the reaction which it exhibits with ferric chloride, the formation of its Cu compound, and its decomposition by nitrous acid, with evolution of N (see general reactions of amido-acids, p. 231) will furnish confirmatory evidence.

Taurine, $C_2H_7NSO_3$

(*β-Amido-ethyl-sulphonic acid*, $H_2N.CH_2.CH_2.SO_2.OH$).

Occurrence. Since taurine was obtained as one of the primary products of the decomposition of taurocholic acid, under the influence of acids and alkalies, it has been shewn, by Limpricht and by Jacobsen, to occur in minute quantities in the muscles of the horse and, by Valenciennes and Fremy, in the muscles of mollusca. It is only, however, by decomposing bile rich in taurocholic acid that it can be obtained in any quantity.

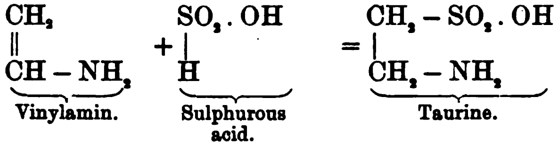
Method of preparation. A little strong hydrochloric acid is added to ox-bile so as to precipitate its mucoid nucleo-albumin. After this has been separated by filtration, a little more strong hydrochloric acid is added and the liquid is then boiled for some hours in a large capsule. Having been allowed to cool, the acid supernatant liquid is decanted from the hard resinoid mass which adheres to the bottom of the capsule, and is further concentrated by evaporation and then set aside, until the greater part of the sodium chloride in solution has crystallised. The cooled mother liquor is then treated with strong alcohol, which causes a separation of taurine mixed with some sodium chloride. The substance which thus separates is washed with absolute alcohol, dried and dissolved in the smallest possible quantity of boiling water, which on cooling deposits taurine in fine four-sided prisms. By repeated crystallisation the substance is obtained free from all traces of sodium chloride[1].

Synthetic formation.

Taurine may be obtained by the action of ammonia on β-chlorethylsulphonic acid.

$$\underbrace{CH_2Cl.CH_2.SO_2.OH_2}_{\beta\text{-chlorethylsulphonic acid.}} + 2NH_3 = \underbrace{CH_2(NH_2).CH_2.SO_2.OH}_{\text{Amidoethylsulphonic acid or taurin.}} + NH_4Cl$$

It may be also obtained by adding an excess of sulphurous acid to a solution of vinylamin and evaporating on the water bath.



$$\underbrace{\begin{array}{l} CH_2 \\ \| \\ CH - NH_2 \end{array}}_{\text{Vinylamin.}} + \underbrace{\begin{array}{l} SO_2 . OH \\ | \\ H \end{array}}_{\text{Sulphurous acid.}} = \underbrace{\begin{array}{l} CH_2 - SO_2 . OH \\ | \\ CH_2 - NH_2 \end{array}}_{\text{Taurine.}}$$

Physical and chemical properties.

Taurine crystallises in colourless shining prisms, which are often large and have four or six sides. It is soluble in from 15 to 16 parts of water at ordinary

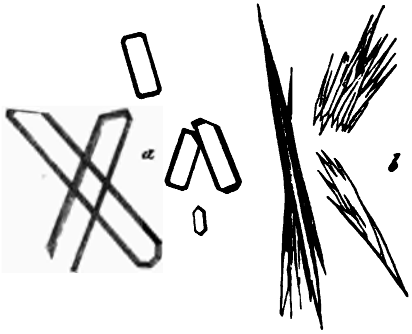


FIG. 22. CRYSTALS OF TAURINE.
(*a*) pure, (*b*) impure.

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[1] Drechsel, *Anleitung zur Darstellung*, &c., p. 35.

temperatures and in a much smaller volume of boiling water. It is insoluble in absolute alcohol and in ether.

Taurine is tasteless and its solutions are neutral to test paper.

When boiled with HCl, or pure HNO_3, it is not decomposed; when, however, it is boiled with a strong alkaline ley it evolves acetic and sulphurous acids, but no alkaline sulphide is formed. When fused with a mixture of nitre and sodium carbonate, the sulphur contained in taurine is oxidised to sulphuric acid, and from the amount of the latter formed the quantity of taurine may be determined.

Metallic salts added to solutions of taurine cause no precipitate. If, however, moist mercuric oxide is gradually added to a boiling solution of taurine a compound of taurine and mercuric oxide falls which is white, almost insoluble in water and quite insoluble in alcohol.

Identification of Taurine. This depends on the crystalline form, a study of the solubility and the determination of the presence and amount of sulphur which the body contains.

SECT. 8. THE BILE COLOURING MATTERS.

Historical Introduction.

The colour of the bile attracted the attention of the physicians of antiquity and even before the days of scientific chemistry some observations, not wanting in accuracy, had been made on the changes which the colour of the bile undergoes under the influence of putrefaction, in the presence of air, and under the influence of acids[1, 2].

Thenard, early in the century, described a yellow colouring matter

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[1] Refer to an account of the bile in Georg Heuerman's *Der Arzney-Gelahrheit Doctors Physiologie*. Dritter Theil. Copenhagen und Leipzig, bei Friedrich Christian Pelt, 1753. "Das merckwürdigste hiebey ist, dass selbige, wie der Herr Seger schon augemercket ('De ortu et progressu bilis cysticae, § 13') durch Beymischung des Spiritus nitri, salis und Olei vitrioli, so besonders ihre Farbe verwandelt, denn mit dem ersten wird es *fast augenblicklich* grün, &c." p. 786. Heuermann's book is one of the most remarkable and instructive of the physiological treatises of the last century, both on account of the acquaintance of its author with the literature of his time and of his philosophical and original views.

[2] Haller in the chapter in which he treats of the action of acids on bile, 'Ut se habeat ad acida,' has some remarks which prove conclusively that long before the time of Leopold Gmelin the action of nitric acid on the biliary colouring matters had been observed, although the 'play of colours' which constitutes 'Gmelin's reaction' had not been described: "Spiritus nitri bilem efficacius cogit, ut virides et duri grumi in sero subsideant. Viridem fecit, quae flava fuerit.......Cum aqua forti alias arbusculae virides natae sunt; et grumus in fundo subsedit. In aliis puto meracioris acidi exemplis, bilis in coagulum amarum, viridis resinae similis, abiit, &c." *Elementa Physiologiae corporis humani*, Auctore Alberto v. Haller. Bernae, Sumptibus Societatis Typographicae, MDCCLXIV. Vide Tom. VI. p. 554.

(Matière jaune de la bile) as characteristic of the bile[1], and L. Gmelin in the researches of which he published the results with Tiedemann[2], studied with some care the properties which this colouring matter presented in the bile of the dog. He shewed that when hydrochloric acid is added to bile confined over mercury its colour undergoes no perceptible change, whilst if oxygen be admitted the bile gradually assumes a green colour. He pointed out that when nitric acid is added to bile, changes in colour occur instantaneously and, under the oxidising influence of this acid, without the intervention of atmospheric oxygen. He discovered that nitric acid occasioned a rapid succession of tints—first green, then blue, violet, and last of all a yellow or yellowish brown—that the very tints of the rainbow present themselves to the observer. "Man versetze Galle mit so viel Salpetersäure, dass die blaue Färbung eintritt, übersättige mit Kali und giesse dann Vitriolöl in hinreichender Menge hinzu, so hat man," he quaintly adds "ein Stück vom Regenbogen." From the date of this description the succession of tints which the bile colouring matters exhibit when treated with nitric acid has received the name of 'Gmelin's reaction.'

It was Berzelius[3] who first attempted the scientific study of the colouring matters of the bile. To the brownish-yellow colouring matter characteristic of the bile of man and the carnivora, he applied the term cholepyrrhin[4], though he confessed himself unable to separate it in a state of purity from the bile itself, studying its properties as observed in solutions, or as he extracted it from the deposits which occurred in the gall bladder or from gall stones.

He described cholepyrrhin as a nitrogenous body of a beautiful reddish-yellow colour, tasteless and without odour, very sparingly soluble in water and alcohol, and most readily dissolved in dilute solutions of caustic potash or soda. He observed that these alkaline solutions of the bile colouring matter absorbed atmospheric oxygen, that the liquid gradually became green, and that when acids were then added to it the colouring matter was precipitated in green flakes. He described this colouring matter as in all respects similar to chlorophyll, the colouring matter of leaves, with which he believed it to be identical. As occurring in the bile he termed it, however, *biliverdin*[5], and he believed that the green colouring matter found in the normal bile of the herbivora was produced from bilirubin by processes occurring within the body which were identical with those which he had studied *in vitro*.

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[1] Thenard, 'Mémoire sur la Bile,' lu à l'Institut le 2 floréal, an 13, in *Mémoires de Physique et de Chimie de la Société d'Arcueil.* Tome I. Paris, 1807, see pp. 23—45.

[2] Tiedemann and Gmelin, *Die Verdauung nach Versuchen*, 1826, p. 79.

[3] Berzelius, see the account which he gives of his researches in his article 'Galle' in Wagner's *Handwörterbuch der Physiologie*, Vol I. p. 522.

[4] From *χόλη*, bile and *πυρρός*, tawny, reddish-yellow (darker than *ξανθός*). The name given by Berzelius appears both on etymological and descriptive grounds preferable to the one which has supplanted it, viz. Bilirubin.

[5] 'Ich habe es in diesem Zustand Biliverdin genannt.' Berzelius, *loc. cit.* p. 522.

Berzelius[1] subsequently separated biliverdin from the precipitate which barium chloride throws down when added to the bile of the ox, and he came to the conclusion that the body thus obtained was also identical with chlorophyll, an error which was perpetuated almost to our own days.

The strictly modern researches on the bile colouring matters may be said to date from the investigations of the colouring matter of gall stones by Heintz[2]; shortly afterwards Valentiner[3] discovered that the body which Berzelius had termed cholepyrrhin, and Simon and Heintz biliphain and which we now term bilirubin, is very soluble in chloroform and that from its chloroformic solution it can be obtained in the form of reddish-yellow microscopic crystals[4]. It is, however, to the thorough researches of Städeler that we owe the most complete investigation of the bile colouring matters[5]. To these and subsequent researches we shall incidentally refer in discussing the individual bile colouring matters.

Bilirubin $C_{32}H_{36}N_4O_6$

(*Synonyms: Cholepyrrhin, Biliphæïn, Bilifulvin, Hæmatoidin*[6]).

Occurrence. Bilirubin occurs in the yellow or reddish-yellow bile of man and carnivorous animals, in the bile of the pig and occasionally in the bile of the herbivora which have been long without food. It also occurs in the contents of the small intestine and is a normal constituent of the blood serum of the horse[7]. It is further a common constituent of gall-stones; it occurs in the urine, and stains the conjunctivae and skin, in cases of jaundice. In old blood extravasations it occurs in microscopic crystals which were first discovered by Virchow and by him called hæmatoidin.

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[1] Berzelius, *Lehrbuch der Chemie*. Uebersetzt von F. Wöhler. 3 Aufl. (Dresden und Leipzig). Vol. IX. (1840) p. 281.

[2] Heintz, 'Ueber den in den Gallen-Steinen enthaltenen Farbstoff.' Poggendorff's *Annalen*, Vol. LXXXIV. (1851), p. 106—116.

[3] Valentiner. Günsberg's *Zeitschrift*. N. Ser. Vol. I. p. 46 (quoted at second-hand).

[4] It appears to have escaped the observation of critical writers on the bile colouring matters that Berzelius was certainly the first to obtain a crystalline colouring matter from the bile of the ox. The process which yielded it was complicated, but its repetition would probably yield interesting results. Berzelius assigned the name of bilifulvin to the crystalline body which he obviously did not consider identical with the impure bilirubin to which he applied the name of cholepyrrhin: "Bilifulvin habe ich eine noch problematische aus *Bilis bubula spissata* erhaltene, krystallisirte rothgelbe Substanz genannt, die ich noch nicht gehörig zu studiren Gelegenheit hatte."

Having described his process of separation he thus expresses himself as to the product: "So bekommt man eine gelbe Lösung, die verdunstet ein rothbraunes Extract zurücklässt. Wird dieses in Alkohol aufgelöst und die Lösung der freiwilligen Verdunstung überlassen, so schiessen daraus zuerst kleine rothgelbe Krystalle an. Diese Krystalle sind es die ich Bilifulvin genannt habe." See Berzelius, *Lehrbuch d. Chemie* (Uebersetz. v. Wöhler), Vol. IX. (1840), p. 285.

[5] G. Städeler, 'Ueber die Farbstoffe der Galle.' *Annalen der Chem. u. Pharmac.* Vol. CXXXII. (1864), p. 323 *et seq*.

[6] The name hæmatoidin is only applied to bilirubin when occurring in old extravasations of blood.

[7] Oloff Hammarstan, 'Om förekomsten af gallföryämm i blodserum.' Upsala, Läkareförening's *förhandlingar*, Vol. XIV. p. 50. See the abstract of this paper, 'Ueber das Vorkommen von Gallenfarbstoff in dem Blutserum,' by its author in Maly's *Jahresbericht*, Vol. VIII. (1879), p. 129.

Methods of separation. In the separation of bilirubin, from whatever source it is obtained, we rely on the remarkable solubility of the free colouring matter in chloroform, whilst its compounds are insoluble in that liquid.

1. *Separation from Gall-stones.* The gall-stones are finely powdered and then thoroughly extracted with ether which extracts the cholesterin which these concretions contain. The residue is then extracted with boiling water, and afterwards treated with dilute hydrochloric acid. The latter decomposes the bilirubin-calcium, the pure bilirubin remaining as an insoluble powder which is thoroughly washed with water, dried and then boiled with chloroform. The chloroformic solution of bilirubin is evaporated on the water bath and the residue is extracted with absolute alcohol and ether. The former solvent separates a certain quantity of a pigment, termed by Städeler, *bilifuscin.* The residue insoluble in alcohol and ether is then again dissolved in chloroform, the solution, if necessary, filtered, and evaporated until bilirubin commences to separate; alcohol is then added to the concentrated chloroformic solution, when an amorphous orange-coloured precipitate of bilirubin, which resembles sulphide of antimony, is obtained. These operations may be repeated. Ultimately the purified bilirubin is once more dissolved in chloroform, and the chloroformic solution allowed to evaporate spontaneously, when crystals of bilirubin separate.

2. *From bile.* (1) Bile, preferably of the dog, is acidulated with acetic acid and shaken with chloroform, care being taken to exclude air as completely as possible[1]. The chloroformic solution is separated, evaporated to dryness, the residue treated with absolute alcohol, and the matter which is left undissolved by this solvent is dissolved in chloroform. By the spontaneous evaporation of the chloroformic solution, crystals of bilirubin may be obtained. The purification of the bilirubin may, if the quantity of material be sufficient, be carried out as directed in the case of bilirubin prepared from gall-stones.

(2) The bile from which bilirubin is to be prepared is diluted with water and precipitated with milk of lime. A solution of carbonic acid is passed through the mixture and the bulky precipitate, having been collected and washed, is suspended in water, decomposed by means of hydrochloric acid and shaken with chloroform, care being taken in these operations to avoid the access of air. The chloroformic solution is evaporated to a very small bulk, pre-

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[1] In all cases in which it is sought to separate bilirubin, so long as the solutions contain free acids or alkalies the greatest care must be taken to avoid the access of air; in the presence of atmospheric oxygen the conversion of bilirubin into biliverdin at once commences, in acid or alkaline solutions.

cipitated with alcohol, and the further purification of the separated bilirubin carried out as already directed[1].

Physical and Chemical Characters.

Colour and crystalline form.

Bilirubin occurs in an amorphous and in a crystalline condition. In the former it presents the appearance of an orange-coloured powder resembling sulphide of antimony; in the latter it has the colour of crystallized chromic acid. Examined under the microscope, crystalline bilirubin exhibits orange-coloured rhombic tables, in which the obtuse angles are often rounded off. When crystallising from solutions which are not quite pure (containing cholesterin, &c.) better formed crystals are obtained than is the case when the solutions contain no such impurities (Hoppe-Seyler[1]).

Solubility.

Bilirubin is insoluble in water, almost insoluble in ether and very sparingly soluble in alcohol. It is readily soluble in chloroform especially with heat; it is likewise soluble (though to a much less extent than in chloroform) in benzol, carbon disulphide, amyl alcohol, and glycerin. These fluids dissolve enough however to acquire a yellow or a brown red colour. Solutions of bilirubin which contain 1 part in 500000 exhibit a perceptible yellow colour when a layer 1·5 cm. thick is observed (Hoppe-Seyler).

Bilirubin is readily soluble in dilute solutions of sodium and potassium hydrate and ammonia, and if the solutions be kept from contact with air or with oxygen, it can be reprecipitated from them by the addition of hydrochloric acid.

It is important to notice that solutions of bilirubin in alkalies do not yield the colouring matter to chloroform. A chloroformic solution of the colouring matter shaken with dilute sodium or potassium hydrate is at once decolourised; on the other hand a similar alkaline solution of bilirubin if acidulated and shaken with chloroform at once gives up its colouring matter, which is dissolved by the chloroform and imparts to it a much less brownish-yellow colour.

Bilirubin forms compounds with bases of which several have been studied. The Na-compound is obtained by precipitating a dark orange solution of bilirubin in sodium hydrate by means of a concentrated solution of caustic soda.

The Ca-compound is obtained by precipitating an ammoniacal solution of bilirubin with calcium chloride. The precipitate is rust-coloured, flocculent, and insoluble in water, alcohol, ether and chloroform. It has the composition indicated by the formula $C_{32}H_{34}N_4O_6.Ca$. When this compound is dried *in vacuo* over sulphuric acid it is of a dark-green colour with a metallic lustre, but when powdered it has a dark-brown colour.

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[1] Hoppe-Seyler, *Physiologische Chemie*, Berlin, 1887, p. 294.

By the action of barium chloride, lead acetate, and nitrate of silver on ammoniacal solutions of bilirubin, compounds similar to the calcium compound can be obtained. The silver compound occurs in violet-coloured flakes and is not reduced even when the liquid in which it is suspended is boiled. Bilirubin, as Maly observes, shews by the compounds which it forms, that it has the character of a weak acid.

Composition and formula. Heintz[1] was the first chemist to make an ultimate analysis of bilirubin, and assigned to it the formula $C_{16}H_{18}N_2O_5$. The method which he followed in the preparation of the substance, which was not until later obtained crystallised, renders it certain that it was not free from impurities, and the results of his analysis may therefore be left out of consideration. The same objection does not apply to Städeler's methods. The results of his work have been absolutely confirmed by the more recent and exhaustive researches of Maly, as well as by Hoppe-Seyler[2]. Both Städeler and Maly from their analyses deduced for bilirubin the formula $C_{16}H_{18}N_2O_3$. Thudichum[3], on the other hand, has assigned to bilirubin the formula $C_9H_9NO_2$, which neither agrees with the concordant analytical results of Städeler and Maly, nor fits in with many facts with which we are acquainted. The reader will see at a glance how considerable are the differences in the percentage of the various elements calculated from Städeler and Maly's formula on the one hand, and from that of Thudichum on the other.

	(Städeler and Maly.)	(Thudichum.)
	$C_{16}H_{18}N_2O_3$ or $C_{32}H_{36}N_4O_6$	$C_9H_9NO_2$
Carbon	67·13	66·25
Hydrogen	6·29	5·52
Nitrogen	9·79	8·59
Oxygen	16·79	19·64
	100·00	100·00

Quite apart from the remarkable concordance of the results of Städeler and of Maly, an examination of all facts bearing on the question[4] has led chemists to the opinion that the formula of

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[1] Heintz, Poggendorff's *Annalen*, Vol. LXXXIV. p. 106.

[2] "Ausser diesen Ergebnissen der Untersuchungen von Städeler sind noch von Maly und von Thudichum solche veröffentlicht, von denen die Resultate Maly's Bestätigung der Untersuchungen Städeler's geben. Die Analysen von Hoppe-Seyler lassen gleichfalls keinen Zweifel an der Richtigkeit der Formel von Städeler und von Maly." Hoppe-Seyler, *Handbuch d. Phys. u. Path. Chem. Analys.*, 6th ed., (1893), p. 226.

[3] Thudichum, *Journ. f. prakt. Chem.*, Vol. CIV. (1868), p. 193.

[4] Such as the results of the analysis of the calcium compound of bilirubin, of Maly's tribromobilirubin, no less than the relation of bilirubin to biliverdin; to the latter point reference will again be made.

Städeler and Maly, or probably a multiple of it, is correct. The various reactions are best explained by doubling Städeler's formula.

Action of nitric acid on bilirubin. 'Gmelin's reaction.'

When bilirubin is treated with pure dilute nitric acid (containing 20 per cent. of HNO_3) no change occurs at ordinary temperatures. When the solution is heated, however, dark-violet resinous flakes are formed which as the temperature rises assume a light-brown colour and ultimately dissolve, yielding a yellow-coloured liquid.

Pure concentrated nitric acid acts in the cold and a cherry-red liquid is obtained which retains its colour for many days. Nitric acid which has a slightly yellow colour and which contains nitrous acid[1] (as the nitric acid of commerce does) gives rise in solutions which contain bilirubin, to a remarkable play of colours already referred to as 'Gmelin's reaction.' The reaction may be tried with a dilute alkaline solution of bilirubin, with diluted bile, or with any liquid, such as the urine of jaundice, which contains bilirubin.

Various methods of exhibiting Gmelin's reaction may be adopted. The most common is to pour some of the solution to be tested into a test tube containing nitric acid, so that the two liquids are not mixed. Near the line of junction the colour-reaction at once commences to develope, and a succession of zones of colour appear, the tints being, from above downwards, as follows:—green, blue, violet, red and reddish-yellow. These tints represent the successive stages of the reaction, the first being the green and the last the reddish-yellow, which is observed in the region where the oxidising action is most intense, viz. in close proximity to the nitric acid.

Instead of employing a test tube, a few drops of diluted bile, or bilious urine may be poured upon a flat plate, so that a thin layer of liquid is obtained. On now adding a drop or two of coloured nitric acid, wherever the acid falls a series of concentric coloured rings of beautiful colour is developed, the succession of tints being the same as in the experiment previously described.

The delicacy of 'Gmelin's reaction' is such that it permits of the detection of bilirubin in solutions which contain only 1 part of the colouring matter in from seventy- to eighty-thousand parts of water. It must be remembered that in order to be sure of the presence of bilirubin the whole series of tints must be observed, as *lutein* (the yellow crystalline matter obtained from corpora lutea, from the yolk of egg, and which is also present in the liquor sanguinis of some animals), when treated with nitric acid, exhibits a green and also a blue tint very similar to those developed in Gmelin's reaction. The spectroscopic characters of lutein are, however, sufficiently distinctive to enable the observer to ascertain whether this substance is present in a solution or not.

Each tint in Gmelin's reaction corresponds apparently to a definite chemical change, probably to a definite oxidation product.

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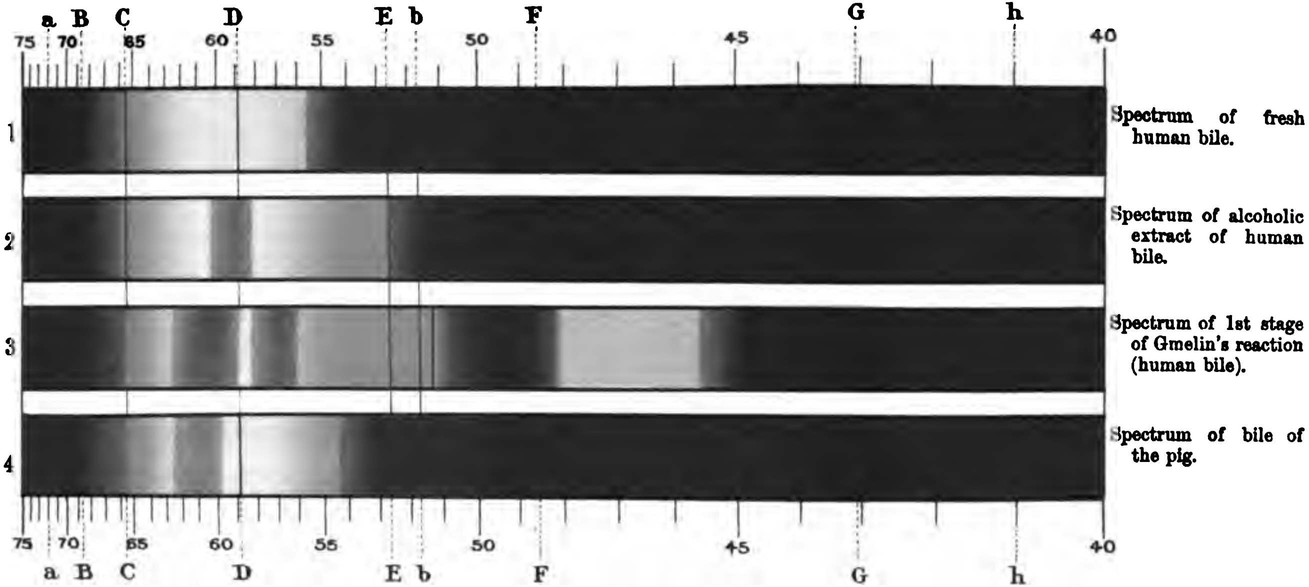
[1] If the acid is too highly coloured (i.e. if the amount of nitrous acid and of nitrogen peroxide be large) it exerts so energetic an action on the bilirubin that the successive stages of Gmelin's reaction cannot be properly observed.

Plate I. Spectra of the Bile of Man, and of the Pig, and of the First Stage of Gmelin's Reaction.

(After MacMunn, with the addition of a scale of wave-lengths. The numbers attached to the scale indicate wave-lengths expressed in 100,000ths of a millimetre. Each division of the scale represents a difference of wave-length of 1·100,000th of a millimetre.)


a
B
C
D
E
b
F
G
h
75
70
65
60
55
50
45
40
1
2
3
4
Spectrum of fresh human bile.
Spectrum of alcoholic extract of human bile.
Spectrum of 1st stage of Gmelin's reaction (human bile).
Spectrum of bile of the pig.

[illegible] production of [illegible] which [illegible] studied [illegible] colour is [illegible]

[illegible] by nitrogen [illegible] following [illegible]

[illegible] compounds [illegible] formed were [illegible] progressive [illegible]

	Carbon	[illegible]	Oxygen
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

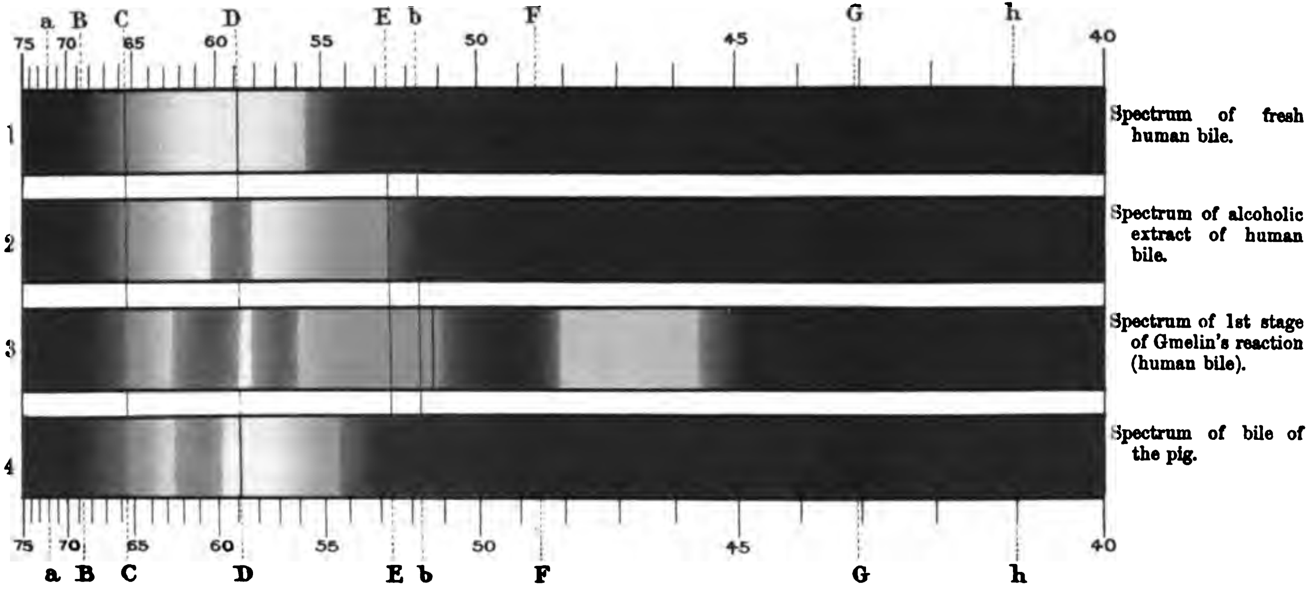
[illegible]

[illegible] reaction. [illegible] 18[illegible] made a series [illegible] with [illegible]

[illegible]

PLATE I. SPECTRA OF THE BILE OF MAN, AND OF THE PIG, AND OF THE FIRST STAGE OF GMELIN'S REACTION.

(After MacMunn, with the addition of a scale of wave-lengths. The numbers attached to the scale indicate wave-lengths expressed in 100,000ths of a millimetre. Each division of the scale represents a difference of wave-length of 1·100,000th of a millimetre.)



the bilicyanin and choletelin stages of Gmelin's reaction. In 1877 Dr MacMunn[1], quite independently of the above researches which were unknown to him, again described with correctness the spectrum of Gmelin's reaction.

The spectrum of the second stage of Gmelin's reaction is shewn in Plate I., Sp. 3.

Ehrlich's reaction.

Gmelin's reaction, with slight modifications to be afterwards noticed, is common to biliverdin and other biliary colouring matters. We have now to speak, however, of a reaction, discovered by Professor Ehrlich[2], which is characteristic of bilirubin and is not exhibited by biliverdin. To a solution of bilirubin in chloroform, is added an equal volume, or twice its volume, of a solution of sulphanilic acid (1 grm. sulphanilic acid, 15 c.c. of hydrochloric acid and 0·1 grm. of sodium nitrite dissolved in distilled water and diluted to 1 litre) and then as much alcohol as is needed to render the solution clear. The liquid, which is of a yellow colour at first, assumes a beautiful red tint. On adding dilute hydrochloric acid, drop by drop, the colour changes first to violet and then to an intense blue. On now carefully pouring into the test-tube a solution of potassium or sodium hydrate, three zones of colour are visible; near the alkaline solution, where the reaction is commencing, the colour is green; at the surface, where the reaction is still acid, the original blue tint persists, whilst intermediate between these two zones is a red, neutral, zone.

The late Professor Krukenberg published a full account of the spectroscopic characters of Ehrlich's reaction. The acid azure blue solution exhibits an absorption band between C and E, of which the centre is a little to the violet side of D, and which as the concentration increases extends more and more towards E. For further details the reader is referred to the original memoir[3].

The action of bromine on bilirubin.

Both Thudichum[4] and Maly[5] have investigated the action of bromine on bilirubin. The following account is based on the observations of Maly. When a solution of bromine in chloroform is added very gradually to a solution of bilirubin in chloroform, a play of colours is observed which is precisely similar to that which constitutes Gmelin's reaction, the colours being the same, as well as the order in which they appear.

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[1] C. A. MacMunn, 'Studies in Medical Spectroscopy,' *Dub. Journ. of Med. Sc.* 1877; see also *The Spectroscope in Medicine*, by the same author, pp. 160—162.

[2] P. Ehrlich, 'Sulfodiazobenzol, ein Reagens auf Bilirubin,' *Centralblatt f. klin. Med.*, Vol. IV. (1883), p. 721; also in *Zeitschrift f. anal. Chemie*, Vol. XXIII. (1883), p. 275.

[3] Dr C. Fr. Krukenberg, 'Das Spectrum der Ehrlich'schen Bilirubinprobe' in Krukenberg's *Chemische Untersuchungen zur wissenschaftlichen Medicin.* Erstes Heft, Jena 1886, p. 77—79. The description is illustrated by drawings of three spectra.

[4] Thudichum, *Journ. of the Chemical Society.* Ser. II. Vol. XIII. p. 389.

[5] R. Maly, 'Untersuchungen über die Gallenfarbstoffe,' *Sitzungsber. d. k. Wiener Akad. d. Wissenschaft*, Vol. LXXII. (1875). A very complete account of this paper is given in Maly's *Jahresbericht*, Vol. V. (1876), pp. 193—198.

The supposition that the coloured bodies obtained under the action of bromine were, like those obtained with a mixture of nitric and nitrous acids, products of oxidation, was disproved by the examination of the green biliverdin-like body which is the first product of the action of bromine. It was at once apparent that this body possessed a green colour because of its being a mixture of undecomposed bilirubin and of a beautiful blue substance. This body was prepared in various ways and was found to have an uniform composition, represented by the formula $C_{32}H_{33}Br_3N_4O_6$. According to Maly the reaction in which it is formed may be represented thus:—

$$\underbrace{C_{32}H_{36}N_4O_6}_{\text{Bilirubin.}} + 3Br_2 = \underbrace{C_{32}H_{33}Br_3N_4O_6}_{\text{Tribromobilirubin.}} + 3HBr.$$

Tribromobilirubin is insoluble in water, readily soluble in alcohol and ether, its solutions in which possess a dark blue colour. The addition of pure acid renders the alcoholic solution more intensely blue than before; this deep blue colour is characteristic of solutions in acetic and glacial acetic acid. Alkaline solutions readily dissolve the body and acquire a violet colour. When boiled with sodium carbonate a green solution is obtained.

The violet alkaline solutions of bromobilirubin, made with the aid of alkaline hydrates and carbonates, at first assume a blue colour when acids are added to them. After some time, however, when acids are added, the colour changes to a pure green. Chlorine readily bleaches the compound.

By the action of sodium amalgam, tribromobilirubin is converted into hydrobilirubin. When digested with solution of caustic soda, dilute sulphuric acid throws down a green body which appears to consist of biliverdin.

Biliverdin $C_{32}H_{36}N_4O_8$.

Occurrence. To the colouring matter which imparts a green colour to the bile of the ox, sheep and other herbivorous animals, the term biliverdin was applied by Berzelius, and it is usually assumed, probably correctly, that this body is identical with the biliverdin which results from the action of various oxidising agents on bilirubin. The strict scientific proof of this identity is, however, not forthcoming, no method having as yet been devised for the separation of pure biliverdin from the bile.

Biliverdin is doubtless the colouring matter found in association with bilirubin in the placenta of bitches; it likewise may be found in vomited matters and in the contents of the small intestine. Biliverdin is, occasionally, a constituent of gall-stones. It is said sometimes to occur in jaundiced urine; its presence in urine which has been exposed to the air affords, however, no proof of its having been present in the fresh secretion.

Preparation. It has already been stated that when the reddish-yellow bile of carnivorous animals is exposed to the air, it gradually acquires a green hue which resembles that of the secretion of the herbivora. This change in colour is associated with an absorption of oxygen and depends upon the conversion of bilirubin into biliverdin; it occurs all the more readily if the bile be rendered decidedly alkaline by the addition of a caustic alkali and then exposed in thin layers to the action of the air. The same change is observed, though it proceeds less rapidly, if the bile be acidified by means of acetic, hydrochloric or sulphuric acids, and then exposed to air; the presence of oxygen being under these circumstances absolutely necessary to the production of the colour. The body which is the cause of the beautiful green tint developed by the action of nitric and nitrous acids upon bile or bilirubin is, unquestionably, biliverdin, which is, in this case, produced by the oxidising action of the acids employed, quite independently of the atmospheric oxygen.

Biliverdin is most readily prepared in a state of purity (Städeler) by dissolving pure bilirubin in a diluted solution of sodium hydrate and either exposing the liquid to air or causing a stream of oxygen gas to pass through it. When the solution has acquired a bright green colour an excess of dilute hydrochloric acid is added; this precipitates the biliverdin which is washed with water, until the washings contain no trace of chlorine. It is then dried, dissolved in absolute alcohol, the alcoholic solution is filtered and precipitated by the addition of water.

Biliverdin is readily produced when bilirubin is heated to 100° C. in sealed tubes containing a mixture of chloroform and glacial acetic acid, care being taken that a considerable space filled with air is left in the tubes (Heynsius and Campbell). The objection to this process is that water very imperfectly precipitates the biliverdin which has been formed. The process was modified in an important manner by Maly[1] who found that at moderate temperatures and in the absence of chloroform, monochloracetic acid readily leads to the absorption of oxygen by bilirubin and its conversion into biliverdin. Monochloracetic acid, the melting point of which is 62° C. is rendered fluid by warming it in a beaker; powdered bilirubin is then digested in it with the aid of a gentle heat, on the water bath After a couple of days, water is added to the dark green solution, the whole of the biliverdin being precipitated. In this process, again, the production of the body is associated with, and dependent upon, the atmospheric oxygen.

Maly[2] also converted bilirubin into biliverdin by the oxidising action of peroxide of lead, the change taking place with extraordinary rapidity. To an alkaline solution of bilirubin some PbO_2 is added and the mixture is stirred. In a couple of minutes the fluid assumes a dark green colour. On now faintly acidifying it with acetic acid, a compound of biliverdin with lead falls. This is decomposed by means of alcohol containing sulphuric acid in solution. The alcoholic solution, filtered from precipitated lead sulphate, is poured into water and the flocculent precipitate of biliverdin is collected.

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[1] Rich. Maly, 'Ueber Biliverdin,' Aus Untersuchungen über die Gallenfarbstoffe, IV. Abhandl., *Sitzungsber. d. Wiener Akademie*, Vol. LXX. (1874). The Author has not seen this paper but quotes from the careful abstract in Maly's *Jahresbericht*, Vol. IV. (1875), pp. 302—304.

[2] Maly, *Sitzungsber. d. Wiener Akad.*, Vol. LVII. (1868). Quoted by Maly in Hermann's *Handbuch*, Bd. VII. p. 158.

Physical and chemical properties of biliverdin.

'Biliverdin is a blackish green solid which is usually amorphous, but which has occasionally been obtained in the form of green rhombic plates with truncated ends, by evaporating its solution in glacial acetic acid. It is insoluble in water, ether, pure chloroform, benzol, and carbon disulphide. It is readily soluble in ethyl alcohol, methyl alcohol, in glacial acetic acid and also in chloroform which contains alcohol, or which has been mixed with glacial acetic acid. It dissolves in concentrated sulphuric acid, forming a solution which is precipitated by water. Strong hydrochloric acid dissolves some biliverdin. Acid solutions of biliverdin—e.g. solutions in glacial acetic acid, are of a beautiful fiery-green colour. Neutral solutions are of a sap-green colour, whilst solutions in alkalies are yellowish-green or brownish-green and are precipitated by acids. Solution of calcium and barium hydrates throw down from alcoholic solutions of biliverdin, flocculent Ba- and Ca-compounds. Solutions of biliverdin exhibit with the spectroscope no definite absorption bands. The absorption increases from the red towards the violet end of the spectrum, so that the extreme red is 16 times less absorbed than the violet between G and H[1].'

Composition of biliverdin and its relation to bilirubin.

Heintz had discovered that when the reddish-yellow colouring matter of the bile is introduced into absorption tubes, containing oxygen and standing over mercury, as the colouring matter assumes a green tint the volume of oxygen diminishes. Städeler, as a result of his analyses, believed that biliverdin differed from bilirubin, as shewn by the following equation:

$$\underbrace{C_{16}H_{18}N_2O_3}_{\text{Bilirubin.}} + H_2O + O = \underbrace{C_{16}H_{20}N_2O_5}_{\text{Biliverdin.}} \text{ (Städeler).}$$

The concordant analyses of Maly and of Thudichum both agree in assigning to biliverdin a formula differing from that of Städeler $(C_8H_9NO_2)_n$. Thudichum, whose formula of bilirubin $(C_9H_9NO_2)$ is obviously incorrect, has expressed the view that when that body is converted into biliverdin it is due to an oxidation which leads to an elimination of CO_2. This view is unquestionably founded on error, and is disproved, firstly by the concordant analyses of pure bilirubin made by Städeler, Maly and Hoppe-Seyler, and secondly by the analyses of biliverdin made by Maly and by Thudichum himself. A comparison of the two sets of analytical results and of the empirical formulæ for bilirubin and biliverdin deducible from them establishes that biliverdin differs from bilirubin only in containing more oxygen.

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[1] Maly, Hermann's *Handbuch*, Vol. VII. p. 159.

A study of the relations of bilirubin to biliverdin, and of tribromobilirubin have led chemists to double the formula originally assigned to bilirubin by Städeler as well as that originally assigned to biliverdin by Maly. The relation of bilirubin to biliverdin is expressed in the equation

$$\underbrace{C_{32}H_{36}N_4O_6}_{\text{Bilirubin.}} + O_2 = \underbrace{C_{32}H_{36}N_4O_8}_{\text{Biliverdin.}}. \quad \text{(Maly.)}$$

SECT. 9. SOME DERIVATIVES OF THE NORMAL BILIARY COLOURING MATTERS.

1. *Hydrobilirubin.*

If the normal bile colouring matters are amenable to the action of reducing agents, their reduction must, without a doubt, be effected in the intestinal canal, where the presence of free hydrogen, the development of sulphuretted hydrogen, &c. shew that conditions exist which are adequate to the reduction of organic bodies. Such was the reasoning which led Richard Maly[1] to commence the investigation of the products of reduction of bilirubin.

Preparation. Bilirubin prepared from the gall-stones of the ox was suspended in water to which was added sodium amalgam in small pieces. At the commencement of the process no gaseous hydrogen was evolved. The suspended bilirubin was soon dissolved by the sodium hydrate resulting from the reaction; after some time the brown solution became gradually lighter in colour and, on being shaken, bubbles of hydrogen gas were evolved. An excess of sodium amalgam being added, the process was allowed to go on at ordinary temperatures for two or three days and then, by the aid of gentle heat, on the water bath, until no further change in the colour of the solution could be observed. The solution, being decanted from the subjacent mercury, was treated with an excess of hydrochloric, or acetic, acid. The addition of acid proved, at once, that the bilirubin had been acted upon, for the liquid assumed a dark garnet-red colour. The greater part of the colouring matter is under these circumstances precipitated in dark red-brown flakes, though a part remains dissolved. In proportion as the precipitate is freed from alkaline chloride by washing, it becomes less soluble, so that when it contains neither chlorine nor fixed residue the washings merely exhibit a pale rose-red tint.

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[1] Maly, 'Untersuchungen über die Gallenfarbstoffe. III. Umwandlung von Bilirubin in Harnfarbstoff,' *Annal. d. Chem. u. Pharm.*, Vol. CLXIII. pp. 77—95.

The precipitated body to which Maly assigned the name of hydrobilirubin is, like the bilirubin from which it is derived, soluble in solutions of ammonia and the alkaline hydrates, from which it is precipitated on the addition of acids. Unlike bilirubin, the reduction product is very readily soluble in alcohol, and its alkaline brown solutions, when concentrated, assume a garnet-red tint on the addition of acids or, if dilute, appear of a rose-red colour. Solutions of hydrobilirubin are incapable of assuming a green tint under the circumstances which cause solutions of bilirubin to become green. Chloroform dissolves hydrobilirubin, acquiring an orange colour, and gives up the pigment to alkaline solutions when treated with these.

Hydrobilirubin has not hitherto been crystallised. It appears to form readily soluble compounds with the alkalies and alkaline earths, and sparingly soluble or insoluble compounds with the heavy metals[1]. From the results of his analyses, Maly assigned to hydrobilirubin the empirical formula $C_{32}H_{40}N_4O_7$, and explains its relation to bilirubin by the following equation:

$$\underbrace{C_{32}H_{36}N_4O_6}_{\text{Bilirubin.}} + H_2O + H_2 = \underbrace{C_{32}H_{40}N_4O_7}_{\text{Hydrobilirubin.}}$$

Abel determined the molecular weight of hydrobilirubin by Raoult's method and obtained results which agree with Maly's formula[2].

When treated with one drop of sulphuric acid and a tiny grain of saltpetre, hydrobilirubin exhibits the variegated tints characteristic of Gmelin's reaction. This reaction of hydrobilirubin may be conveniently referred to as '*Liebermann's reaction*,' after its discoverer[3].

Amongst the most striking characteristics of hydrobilirubin are its properties of fluorescence, when treated with zinc chloride, and its absorption spectrum under various conditions.

Whereas solutions of bilirubin and biliverdin exhibit no absorption bands, acid (red) solutions of hydrobilirubin exhibit a dark band between *b* and *F*, which fades on the addition of ammonia, but becomes again much darker, and shifts a little towards the red end, when the ammoniacal solution is treated with a couple of drops of zinc chloride. The zinc chloride solution, when examined by transmitted light, is of a rose-red or a garnet-red colour according to concentration, and exhibits a beautiful green fluorescence which disappears on the addition of acids and reappears on the addition of ammonia.

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[1] For a more recent research on Hydrobilirubin than Maly's consult the following: Ludwig Disqué, 'Ueber Urobilin' (aus dem physiol.-chem. Institut zu Strassburg). *Zeitschr. f. phys. Chemie*, Vol. II. (1879), pp. 259—272.

[2] John J. Abel 'Bestimmung des Moleculargewichtes der Cholalsäure, des Cholesterins und des Hydrobilirubins nach dem Raoult'schen Methode' (Nencki's Laby. Bern.). *Monatsch. f. Chemie*, Vol. XI. (1891), pp. 61—70.

[3] Leo Liebermann, 'Ueber Choletelin und Hydrobilirubin,' Pflüger's *Archiv*, Vol. X. (1874), p. 246.

Vierordt[1] has examined hydrobilirubin spectro-photometrically both when dissolved in alcohol and in a solution of ammonia. In the case of the former the maximum absorption is between λ519·5 and λ501·2; in the case of the latter between λ501·1 and λ486·1[2].

Biliary Urobilin (?).

Jaffé[3] by treating the bile of the dog or its alcoholic extract with dilute hydrochloric acid, obtained a reddish-yellow filtrate which possessed spectroscopic characters which resembled those of a pigment which he found in normal and pathological urines and to which he subsequently[4] ascribed the name of urobilin. MacMunn[5] by treating the bile of various animals (man, pig, ox, sheep, mouse) with alcohol and acetic acid, filtering, diluting the filtrate with water and shaking with chloroform observed that an orange solution is sometimes obtained. He evaporated this coloured solution on the water bath and extracted the residue with rectified spirit. The solution possessed two bands very like those of hydrobilirubin. With ammonia and zinc chloride it assumed a red colour which on exposure to air exhibited a green fluorescence. This pigment is according to MacMunn probably formed in the liver from hydrobilirubin-like products carried to it from the intestine by the portal blood.

It is well to point out, however, that the bile of no animal during life or immediately after death exhibits characters which entitle us to admit the existence of a biliary urobilin and that by the methods employed by Jaffé and by MacMunn secondary products are formed which we have no right to class amongst proximate principles. Of such a nature is, without a shadow of doubt, biliary urobilin.

The subject of urobilin, or rather of the urobilinoid bodies, will be considered in connection with the urinary pigments. In this place it will be merely remarked that according to the majority of physiological chemists, these bodies which have never been obtained in a pure condition or, in a strictly scientific sense, proved to be individual substances are, if not identical with, yet immediately related to, the product which Maly obtained by the action of reducing agents on bile, and to which he ascribed the name of hydrobilirubin. With this view the Author entirely concurs. MacMunn who has paid great attention to this subject, is of a different opinion.

It appears, however, inadmissible to draw far-reaching conclusions as to the existence, origin, identity and relations of complex organic bodies merely from the study of the absorption spectra, or rather of the absorption bands, of organic fluids or extracts, and of the changes which they exhibit under the influence of certain reagents. Such a study should only afford hints for investigations to be conducted by the recognised methods of chemical investigation. It is only by a spectro-photometric study of a large number of spectral regions that the identity of, or the optical differences existing between two bodies can be established. So great are the modifications in light absorption introduced by comparatively trivial circumstances that the greatest caution should be exercised in concluding as to differences between otherwise related bodies on the ground of some variation in their powers of absorbing light.

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[1] Vierordt, 'Die Anwendung des Spectralapparates zur Photometrie der Absorptionspectren.' Tübingen, 1873.

[2] The original observations of Vierordt on the absorption spectra of hydrobilirubin are reprinted in the work entitled *Kolorimetrie und quantitative Spectralanalyse in ihrer Anwendung in der Chemie* von Dr Gerhard Krüss, a. o. Professor der Chemie in der Kgl. Universität in München und Dr Hugo Krüss in Hamburg. Hamburg and Leipzig, 1891. Refer to pages 214 and 215.

[3] M. Jaffé, 'Beitrag zur Kenntniss der Gallen und Harnpigmente,' *Centralblatt f. d. med. Wissenschaft*, Vol. VI. (1868), pp. 240—245.

[4] Jaffé, 'Zur Lehre von den Eigenschaften und der Abstammung der Harnpigmente,' Virchow's *Archiv*, Vol. XLVII. (1869), pp. 405—427.

[5] MacMunn, *Proceedings of the Royal Society*, 1880, No. 208.

Bilicyanin.

It has already been stated that when bilirubin is subjected to the oxidising action of nitric and nitrous acids, one stage in the reaction which ensues (Gmelin's reaction) is characterized by a beautiful blue colour and by a somewhat characteristic absorption spectrum. Several observers have attempted to separate the body upon which the blue colour depends and, although it has never yet been obtained in a state to admit of scientific research, it has already received various names.

Preparation.

Städeler[1] was the first to attempt to separate this colouring matter. He added concentrated nitric acid, containing some nitrous acid, drop by drop, to a dilute ammoniacal solution of bilirubin, adding from time to time ammonia in quantity sufficient nearly to neutralise the excess of acid; there is thus obtained a green flocculent precipitate which gradually turns blue. After washing with water, the green pigment is extracted by means of alcohol, which leaves a dark blue powder undissolved. The quantity of this substance obtained by Städeler did not permit of its investigation.

Jaffé[2] modified Städeler's method somewhat. An alcoholic solution of biliverdin, or a mixed ammoniacal and alcoholic solution of bilirubin, is treated exactly as Städeler recommended. As soon as the blue colour has been developed, the liquid is mixed with chloroform and distilled water and shaken. The blue chloroform solution is repeatedly shaken with distilled water, any biliverdin which has separated is filtered off, and the chloroform solution allowed to evaporate spontaneously. The residue is freed from traces of biliverdin by repeated solution in chloroform.

Stokvis[3], and afterwards Heynsius and Campbell[4], attempted to separate the blue colouring matter and described its spectroscopic characters. Stokvis named it, in the first instance, choleverdin and afterwards cholecyanin, whilst Heynsius and Campbell[1] applied to it the term bilicyanin by which it is now known.

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[1] Städeler, 'Ueber die Farbstoffe der Galle,' *Annalen der Chemie u. Pharm.* Vol. cxxxii. (1864), p. 333.

[2] Jaffé, 'Zur Kenntniss der Gallen und Harnpigmente,' *Centralblatt f. d. med. Wissenschaft*, 1868, p. 242.

[3] B. J. Stokvis, 'Ueber Gallenfarbstoffe,' *Ber. d. d. Chem. Gesellsch.* Berlin, 1872, p. 583. Maly's *Jahresbericht*, Vol. ii. 239; 'Das Gmelin'sche (blaue) Oxydationsproduct der Gallenfarbstoffe.' *Centralblatt f. d. med. Wissenschaft*, 1872, no. 50. Maly's *Jahresbericht*, Vol. ii. p. 239.

[4] Heynsius and Campbell, 'Die Oxydationsproducte der Gallenfarbstoffe und ihre Absorptionsstreifen,' Pflüger's *Archiv*, Vol. iv. (1871), pp. 529 *et seq.*

Physical and chemical properties of bilicyanin.

The product which we are now considering has, doubtless, not been obtained in a pure condition and has never been subjected to analysis. It is not therefore surprising that the description of its properties, given by the various persons who have investigated it, should differ materially. As has already been stated, according to Städeler, bilicyanin presents the appearance of a blackish-blue powder. According to Jaffé, if freed from every trace of acid it is not blue but dark violet. It is insoluble in water but readily soluble in alcohol, ether and choroform, imparting to these liquids a beautiful violet colour which is changed into a lovely blue on the addition of a trace of acid.

Acid solutions of bilicyanin present two absorption bands which are identical with those which are seen during the first stage of Gmelin's reaction and which are situated on either side of the D line (see Plate I., Spect. 3). The band between *b* and *F* which can be seen in the spectrum of Gmelin's reaction is not due to bilicyanin, as was supposed by Heynsius and Campbell, but to the more oxidised product, choletelin. According to Jaffé, neutral and alkaline solutions of bilicyanin exhibit no absorption bands. According to Heynsius and Campbell, they present equally strong absorption bands, though their position is different; they are somewhat shifted towards the red end in the case of the neutral and alkaline, as compared with the acid, solutions.

Nature and relation of bilicyanin.

Bilicyanin appears, unquestionably, to be a product, or a mixture of products, resulting from the moderate oxidation of bilirubin and biliverdin. This oxidation, if carried further, furnishes the body to be subsequently described as choletelin. Bilicyanin occurs occasionally, in small quantities, in gallstones.

Choletelin[2] ($C_{32}H_{36}N_4O_{12}$?).

The name choletelin was given by Maly[3] to the product, or mixture of products, which results from the prolonged action of nitric and nitrous acids on the bile colouring matters, the formation of which coincides with the final (yellow) stage of Gmelin's reaction, which is, therefore, now often designated the choletelin stage.

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[1] The reader who is specially interested in bilicyanin is referred to the table of spectra which accompanies the previously cited paper of Heynsius and Campbell.

[2] Derived from χολή, bile and τέλος, the end.

[3] Maly, *Sitzungsber. d. Wiener Akad.* Vol. LVII. 2 Abth. Febr. 1868: Vol. LIX. 2 Abth. April, 1869. The account here given of choletelin is taken from the account given by Maly, Hermann's *Handbuch*, Vol. VII. p. 165.

Preparation. Bilirubin is suspended in alcohol and the mixture treated with nitrous acid, evolved by the action of nitric acid on arsenic. The liquid assumes successively all the colours of Gmelin's reaction, the bilirubin dissolves and a clear liquid is obtained of a yellowish red colour and possessed of slight tinctorial power. When this liquid is poured into water, choletelin separates in the form of flakes having the colour of ferric oxide; these, when dried, furnish a brown powder. Choletelin has not been crystallised; it is soluble in alkaline solutions, from which it is precipitated on the addition of acids; it is soluble in chloroform, alcohol, ether and acetic acid.

Acid solutions containing choletelin, as for example the yellow liquid which is obtained in the final stage of Gmelin's reaction, exhibit an absorption band between *b* and *F*; this absorption band is generally visible at the same time as the two bilicyanin bands, though it does not, as Heynsius and Campbell believed, belong to that substance.

According to the observations of Maly and of Liebermann[1], neutral alcoholic solutions of choletelin exhibit no definite absorption band, and the accuracy of their statements is placed beyond doubt by the very complete spectro-photometric determinations of Vierordt[2], who made the interesting additional observation that an alkaline solution of biliverdin left to itself for 56 days yielded results on spectro-photometric analysis, which corresponded exactly with those which would be yielded by a solution containing a mixture of pure biliverdin and choletelin.

Relations of choletelin to the bile colouring matters. We have already referred to the fact (p. 320) that the action of nitric and nitrous acids on bilirubin, as determined by a comparison of the elementary composition of bilirubin, biliverdin and choletelin, is one of progressive oxidation and that the amount of nitrogen remains unchanged.

	C	N	O
Bilirubin contains	67·1%	9·8%	16·8%
Biliverdin ,,	63·6,,	9·3,,	21·2,,
Choletelin ,,	55·5,,	9·1	30·0,,

Relying on the very misleading fact that the band observed in acid solutions of choletelin occupies very nearly the position of the well-marked band of hydrobilirubin, Stokvis, as well as Heynsius and Campbell, expressed the belief that the products of the action of nascent hydrogen, on the one hand, and of nitric and nitrous acids, on the other, were identical—in other words that choletelin and hydrobilirubin were one and the same substance. Heynsius accounted for the same body being produced, under such opposite circumstances, on the hypothesis that it was a product not of oxidation or of reduction, but of the splitting-up of the molecule of bilirubin or biliverdin: it being true that the decomposition of some very complex substances sometimes occurs under circumstances which are widely different. Liebermann shewed, however, that when he converted bilirubin into hydrobilirubin the product amounted to 95% of the bilirubin employed, whilst when he converted it into choletelin, the product obtained only represented 72·$\frac{9}{0}$ of the bilirubin employed. A comparison of the elementary composition of bilirubin, hydrobilirubin and of choletelin at once demonstrates the wide difference which exists between the two latter substances as well as in their relations to bilirubin.

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[1] Leo Liebermann, 'Ueber Choletelin und Hydrobilirubin," Pflüger's *Archiv*, Vol. x. (1874), p. 246.

[2] K. Vierordt, 'Physiologische Spectralanalysen,' *Zeitschrift für Biologie*, Vol. x. (1874), pp. 21—58 and Vol. x. 399—409. A very complete abstract of the part of this paper which deals with the spectro-photometry of the bile-colouring matters is given in Maly's *Jahresbericht*, Vol. iv. p. 76—85. A table giving the spectro-photometric constants of choletelin is to be found in Krüss's 'Kolorimetrie, &c.' p. 221.

COMPOSITION OF BILIRUBIN, CHOLETELIN AND HYDROBILIRUBIN.

	Bilirubin	Choletelin	Hydrobilirubin
Carbon	67·1	55·5	64·9
Hydrogen	6·3	5·4	6·7
Nitrogen	9·8	9·1	9·5
Oxygen	16·8	30·0	18·9

The following tabular statement brings out very clearly the points of difference in physical characters between hydrobilirubin and choletelin.

	Hydrobilirubin.	Choletelin.
Produced from bilirubin.	By reduction.	By oxidation.
The spectrum of a *neutral* alcoholic solution.	Exhibits an absorption band between λ501·2 and λ486·1.	No absorption band. The absorption of light between λ501·2 and λ486·1 is $\frac{1}{11}$th of that observed in the case of hydrobilirubin, *cœteris paribus* (Vierordt).
Colour of an acid solution.	Garnet-red to rose-red, according to concentration.	Yellow.

	Hydrobilirubin.	Choletelin.
Colour of alkaline solution.	Brownish-yellow to yellow, according to concentration.	Yellow.
Fluorescent properties of an ammoniacal solution containing zinc.	Exhibits a green fluorescence.	No fluorescence.
Reaction when treated with one drop of sulphuric acid and a tiny grain of saltpetre (Liebermann).	Exhibits the variegated colours characteristic of Gmelin's reaction.	None.

Sect. 10. Imperfectly investigated Colouring Matters not preexistent in Bile, but derived from Chromogens existing in it.

1. *Cholohæmatin.*

When the bile of the sheep or of the ox is examined some time after death, especially after it has been well shaken with air, it usually presents an absorption spectrum which is characterised by the presence of four absorption bands (see Plate II. fig. 1) the position of which will, in the sequel, be indicated.

Hoppe-Seyler's observations.

In the third edition of his practical handbook, published in 1870, Hoppe-Seyler[1] speaking of the bile of the ox described it as possessed, when fresh, of a green colour and exhibiting, when tolerably thick layers are examined with the spectroscope, an absorption band between *D* and *E*, but nearer *D*. He, however, asserted that when such bile is kept it exhibits a spectrum, also observed in the case of the alcoholic solution of evaporated ox-bile, marked by 4 absorption bands, of which one is close to *C*, a second between *C* and *D* but closer to *D*, a third between *D* and *E* but closer to *D*, and a fourth in the proximity of *E*. This author remarked that the colouring matter which occasions this spectrum is also found in sheep's bile, but that nothing was known in regard to it.

The observations of Heynsius and Campbell.

In 1871, in their already quoted paper, Heynsius and Campbell gave an admirable and in all respects accurate plate[2] of the spectrum observed on examining an alcoholic solution of ox-bile. They made the most important assertion that the bile of the ox in the *fresh condition* presents no absorption bands, and that even the alcoholic extract only presents bands after exposure to air.

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[1] F. Hoppe-Seyler, *Handbuch der phys. u. path. chemisch. Analyse*, Dritte Aufl. Berlin, 1870, p. 182.

[2] A. Heynsius and J. F. F. Campbell, 'Die Oxydationsproducte der Gallenfarbstoffe

MacMunn's observations.

In 1880 MacMunn[1], obviously unacquainted with the previous observations, described the bile of the ox and sheep as follows:—"When obtained fresh it is green, but soon changes to reddish-brown, and presents exactly the same spectrum when obtained from the ox that it does when it is got from the sheep. This spectrum is a very fine one, and presents in a deep layer three bands, in a thinner one four bands, and in a still thinner a fifth band at *F* is visible."

Subsequently MacMunn published more detailed descriptions of the colouring matter which occasioned the peculiar spectrum in the bile of the ox and sheep[2] and assigned to it, because of its supposed genetic relationships, the name of cholohæmatin[3]; to MacMunn's researches we shall again refer.

The Author's observations. Cholohæmatin non-existent in the bile at the moment of death.

The reader will have remarked the great discrepancy between the results of the three sets of researches referred to and it appeared to the Author desirable to determine whether the so-called cholohæmatin ever occurs in the bile at the moment of death. In order to settle the point he completely filled sterilised pipettes with bile collected from the gall-bladders of oxen and sheep immediately after the animals had been killed, and at once sealed the pipettes in a flame, taking care to occlude no air. At the same time samples of the same bile were collected in stoppered glass bottles, care being taken that the liquid only partially filled them. These bottles were then shaken so as thoroughly to mix their liquid and gaseous contents.

From a large number of observations it resulted that the bile when obtained from the gall-bladder of the ox or sheep, without coming into contact with air, does not exhibit the spectrum of cholohæmatin, though in thin layers a somewhat indefinite absorption band, having its centre at about λ490, is visible. When, however, such bile is shaken with, or exposed to, air, within an hour, the absorption bands on either side of *D* commence to appear, the one between *D* and *E* being the darker and more easily recognisable. It is very much later that the complete spectrum of cholohæmatin is visible, *i.e.* that the bands near *C* and between *E* and *b* make their appearance.

It is, however, to be noted that the change which occurs in the colour of the bile and in its spectroscopic appearances is not

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und ihre Absorptionsstreifen' (with plate), Pflüger's *Archiv*, Vol. IV. see 540 and 541 and spect. 12 (alc. ext. von fel tauri inspissat.).

[1] MacMunn, *The Spectroscope in Medicine*, London, 1880, see p. 158.

[2] MacMunn, *Proceedings of the Royal Society*, 1883, no. 226.

[3] MacMunn, 'Bile Pigments and Others,' *Journal of Physiology*, Vol. VI. pp. 22 *et seq*, see p. 28.

connected with putrefaction, but commences as soon as the bile is brought in contact with the atmospheric oxygen.

From these observations it is obvious that we have no more right to say that cholohæmatin exists in the bile of the ox or sheep than to assert that fibrin exists in the living blood. The bile of these animals at the time of death contains a chromogen, or chromogens, which under the influence of oxygen gives rise to a body, or bodies, which confer upon the bile the spectroscopic characters studied in the first instance by Hoppe-Seyler and by Heynsius and Campbell, and afterwards by MacMunn. There is no evidence that the four-banded spectrum is due to one substance. It may be, and probably is, due to more than one substance; this view is at least rendered probable by the fact, already referred to, that at one stage in the development of the final product, or products, the spectrum presents only the two central bands, and that the others are superadded as the process of change proceeds.

The Author wishes it to be understood that he does not assert that the so-called cholohæmatin is necessarily a product of oxidation of chromogen or chromogens, in the same sense in which biliverdin is a product of oxidation of bilirubin; it may be a mere product of a decomposition which is initiated by oxidation.

MacMunn's method of separating cholohæmatin[1].

Ox-bile is treated with absolute alcohol, a few drops of acetic acid are added, the liquid is filtered and shaken with chloroform. The orange-coloured chloroform solution is separated, filtered and evaporated to dryness. On dissolving the chloroform extract in ether, a greenish solution is obtained; this is evaporated to dryness, the residue dissolved in chloroform and washed again in a separating funnel with water. "On separating off the chloroform, filtering and evaporating the solution, an amorphous-looking residue of a dark sap-green colour was obtained, which still had a peculiar musky odour. On dissolving some of this residue in alcohol and adding ether no precipitate formed, shewing that bile salts could not have been present."

It is obvious that such a process could yield no pure substance, and that the question whether any individual body exists to which the name cholohæmatin can be applied remains an entirely open one, to be settled by future researches.

MacMunn's description of the spectrum of cholohæmatin.

The spectrum of cholohæmatin is shewn in Spect. I. of Plate II. MacMunn has given the following data as to the approximate position of the four bands of cholohæmatin, expressed in wave-lengths.

1st band centre at λ649.
2nd band from λ613 to 585.
3rd band from λ577·5 to 561·5.
4th band from λ537 to 521·5.

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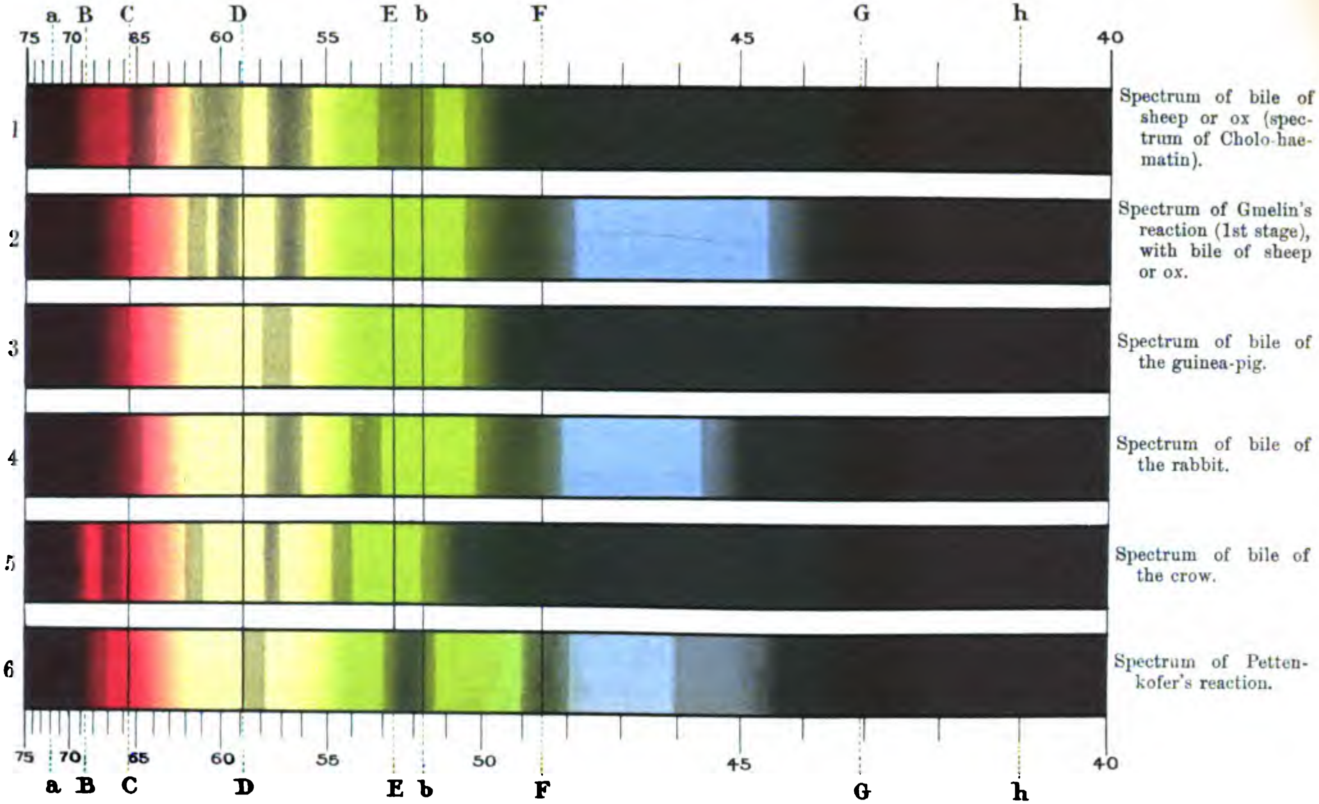
[1] MacMunn, *op. cit.*, *Journal of Phys.*, Vol. VI. pp. 25 and 26.

PLATE II. THE SPECTRA OF CHOLO-HAEMATIN (BILE OF THE OX AND SHEEP); OF GMELIN'S REACTION, WITH BILE CONTAINING CHOLO-HAEMATIN; OF THE BILE OF GUINEA-PIG, RABBIT, AND CROW; OF PETTENKOFER'S REACTION.

(After MacMunn, with the addition of a scale of wave-lengths. The numbers attached to the scale indicate wave-lengths expressed in 100,000ths of a millimetre. Each division of the scale represents a difference of wave-length of 1-100,000th of a millimetre.)



Hoppe-Seyler's and MacMunn's views on the nature of cholohæmatin.

Hoppe-Seyler has enunciated the view that the spectrum of the so-called cholohæmatin is due to the formation, by a process of oxidation, of bilicyanin[1]. Mac Munn very correctly points out the erroneous nature of this explanation[2]. The latter author, upon several grounds, but especially from the fact that by the action of sodium amalgam on his cholohæmatin he obtained a body with an absorption spectrum very closely resembling that of hæmatoporphyrin, argues that cholohæmatin is a derivative of hæmatin. This view in so far as the normal bile-colouring matters, bilirubin and biliverdin, are concerned, is the one which has been generally held and is probably correct. What the relations of the hypothetical individual (if it be one) cholohæmatin to the other bile-colouring matters may be, is a question concerning which we possess no information, and the solution of which requires a thorough chemical investigation, such as those by which Heintz, Städeler, and Maly gradually evolved our knowledge of bilirubin and biliverdin. The researches of MacMunn have, however, served the useful purpose of drawing very particular attention to the subject.

Bilifuscin and *bilihumin* are two products, probably derivatives of bilirubin and biliverdin, which are found in gall-stones, and which will be briefly treated of in connection with these concretions. *Biliprasin* is the name given by Städeler to a colouring matter which he found in the gall-stones of the ox and which is now believed to be a mixture of biliverdin and bilihumin (*q. v.*).

SECT. 11. THE MUCOID NUCLEO-ALBUMIN OF THE BILE.

It has already been stated that the bile, as it flows from the smaller hepatic ducts, is a non-viscid liquid, of which the specific constituents are the salts of the bile acids and the bile-colouring matters, but that by admixture with the secretion of the glands situated in the mucous membrane which lines the gall-bladder and the excretory ducts, it assumes a viscidity which is most marked when the bile has sojourned longest in the gall-bladder.

The viscid constituent of the bile formerly believed to be mucin.

Berzelius was the first chemist to study the body which conferred upon the bile its viscous character and he came to the conclusion that this body was mucin—a view which was held until lately. The researches of Landwehr, published since the 1st volume of this book appeared, have established that the true mucins yield, as essential products of their decomposition, when boiled with dilute mineral acids or when subjected to the action of superheated steam, an albuminous body and a carbohydrate[1]. Landwehr, experimenting on the so-called mucin of bile, observed that when boiled with dilute acids it did not, like the mucin of the salivary glands or the mucin of *Helix pomatia*, yield a substance which exerts a reducing action, and he advanced the hypothesis that in all probability it consisted of a mixture of globulins with bile acids[2], an hypothesis which has however been disproved.

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[1] Hoppe-Seyler, *Handbuch f. phys. u. path. chem. Analyse*, 6te Aufl. Berlin, 1893, p. 226.

[2] MacMunn, *Proc. Royal Soc.* 1883, No. 226, and *Journ. of Phys.* Vol. VI. p. 26.

Fundamental distinction between 'the mucins' and 'the nucleo-albumins[3].'

The researches of Hammarsten[4] and others have made us acquainted with a class of albuminous bodies widely distributed throughout the protoplasmic structures of animal bodies and typically represented by casein, bodies which have been associated together as a family of the albuminous substances under the name of the nucleo-albumins. Just as it is characteristic of the mucins to split up, when subjected to certain hydrolytic agencies, into a proteid and a carbohydrate moiety, so is it a characteristic of a nucleo-albumin to split up, under the same conditions, into a proteid and into a phosphorus-containing nuclein, for all the nucleo-albumins contain phosphorus as an essential element of their molecule.

The researches of Paijkull.

Working under the direction of Hammarsten, Paijkull[5] has separated the body which confers upon bile its viscidity and has conclusively proved that it belongs to the nucleo-albumins, resembling in its characters the mucin-like nucleo-albumin which Hammarsten discovered to be a constituent of the synovial fluid.

Methods of separation of the mucoid nucleo-albumin of bile.

The methods which were formerly employed to precipitate, what was termed, the mucin of bile are not available for the separation of the body. Acids precipitate not only the nucleo-albumin but also bile acids, and the latter cannot be separated by alcohol from the former without rendering the nucleo-albumin insoluble.

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[1] H. A. Landwehr, 'Untersuchungen über das Mucin von Helix pomatia und ein neues Kohlenhydrat (Achrooglycogen) in der Weinbergschnecke,' *Zeitschr. f. phys. Chemie*, Vol. vi. (1882), pp. 74—77; 'Ein neues Kohlehydrat (thierisches Gummi) im menschlichen Körper,' *ibid.* Vol. viii. (1883—4), pp. 122—128.

[2] H. A. Landwehr, 'Ueber Mucin, Metalbumin und Paralbumin,' *Zeitschr. f. phys. Chemie*, Vol. viii. (1883—4), pp. 114—121, see p. 117.

[3] So far as the Author has been able to discover Hammarsten first suggested the propriety of recognising a group of *nucleo-albumins*, and first introduced this term, in the following sentence: "Zu den gewöhnlichen, künstlich darzustellenden Alkali- oder Kalk-albuminaten kann das Casein keineswegs gerechnet werden; und wenn man es nicht zu einer besonderen Gruppe von Stoffen, den Nucleoalbuminen rechnen will—was wohl das Richtigste sein würde—muss wohl das Casein mit dem grössten Rechte einen Platz unter den nativen Albuminaten, d. h. den Globulinen, finden." Olof Hammarsten, 'Zur Kenntniss des Caseins und der Wirkung des Labfermentes.' Upsala, 1877, p. 75; consult p. 45.

[4] O. Hammarsten, 'Studien über Mucin und mucin-ähnliche Substanzen,' Pflüger's *Archiv*, Vol. xxxvi. p. 373.

[5] L. Paijkull, 'Ueber die Schleimsubstanz der Galle,' *Zeitschrift für phys. Chemie*, Bd. xii. (1887), p. 196.

Two methods are available for the separation of the nucleo-albumin in a pure and unaltered condition, of which the second is the best:—

Firstly. Bile is subjected to dialysis in running water for many days, thymol being added to prevent putrefaction; in this way the bile acids and the greater part of the bile colouring matters are got rid of. The contents of the dialyser, which possess a neutral reaction, a pale yellow colour, and are opalescent and somewhat viscid, are precipitated by the addition of a few drops of hydrochloric acid. The precipitate is washed, dissolved in the smallest available quantity of solution of sodium hydrate and the solution is purified by dialysis.

Secondly. Bile, which must have previously been filtered, is mixed with five times its volume of absolute alcohol and, immediately thereafter, is centrifugalised. In 10 minutes the precipitate, which has aggregated into a coherent mass, is taken from the tubes, freed from adhering liquid by pressing between layers of filter paper, and is then broken up and suspended in water. It gradually dissolves, yielding an opalescent, greenish-yellow, slimy liquid. In order to purify it further, it is once, or even twice, again precipitated as above, centrifugalised, and dissolved in water, the ultimate solution being viscous; it is, however, an essential condition to success that the substance should remain in contact with alcohol for as short a time as possible.

Reactions of a solution of the nucleo-albumin.

The neutral viscid solution does not coagulate on boiling, but becomes opaque. If a trace of acetic acid not sufficient to produce a precipitate be, however, added to it and the solution be then heated, an albumin like coagulum is obtained. When acetic acid is added to the solution of nucleo-albumin at ordinary temperatures, it produces a precipitate which is soluble, though with some difficulty, in an excess of the precipitant. In the presence of salts of the bile acids, the precipitate contains considerable quantities of the latter and is not soluble in excess of acetic acid. Thus is explained the fact that the precipitate of the nucleo-albumin, obtained by treating bile with acetic acid, is insoluble when the latter is added in excess, whilst it is obvious that all determinations of the nucleo-albumin made in this manner cannot be relied upon.

A solution of the nucleo-albumin in acetic acid is precipitated by potassium ferrocyanide, iodohydrargyrate of potassium, mercuric chloride, and tannic acid. Aqueous solutions are precipitated when treated with a very small quantity of hydrochloric acid, the flocculent precipitate, thus produced, being readily soluble in excess of hydrochloric acid. The aqueous solution is precipitated by all the general reagents which precipitate the albuminous substances.

The following reactions are of special importance, as distinguishing the mucoid nucleo-albumin from the other groups of albuminous substances, on the one hand, and from the mucins, on the other:

1st. The solution in 0·3 p% HCl may be long heated to 40° C. without giving a precipitate. If however pepsin be added to the acid solution, heated to 40° C., a flocculent precipitate soon separates. This behaviour, towards hydrochloric acid and hydrochloric acid and pepsin, is characteristic of the nucleo-albumins as a class. 2nd. The nucleo-albumin when boiled for hours with dilute mineral acids yields no substance possessing the power of reducing Fehling's solution. This behaviour distinguishes the nucleo-albumins from the mucins. 3rd. When the purified and dried nucleo-albumin is fused with a mixture of potassium hydrate and saltpetre, the fused mass is found to contain phosphoric acid, and in such proportion as to prove that phosphorus must have been present in an oxidised form; for the quantity of phosphoric acid is greater than could exist in combination with bases, even assuming the entire ash obtained on igniting the nucleo-albumin to consist of calcium phosphate.

It has been stated that the mucoid nucleo-albumin of the bile is derived from the mucous membrane of the gall bladder and biliary passages, an assertion which is proved by the fact that by treating the aqueous extract of the mucous membrane with an excess of acetic acid, a substance is precipitated having the same characters and the same elementary composition as the nucleo-albumin obtained from the bile, by the previously described methods.

Results of elementary analysis.

The following are the results of the analyses of two specimens (No. 1 and No. 2) of the nucleo-albumin prepared from the bile, and of one specimen (No. 3) prepared from the mucous membrane of the gall bladder.

	No. 1.	No. 2.	No. 3.	Mean.
Carbon	—	50·87	50·91	50·89
Hydrogen	—	6·74	6·73	6·735
Nitrogen	16·10	16·09	16·22	16·14
Sulphur	1·58	1·74	1·64	1·66
Ash	0·40	0·73	1·36	

As the substance contained phosphorus in combination with calcium and iron, in addition to phosphorus in organic combination, the amount of the latter could not be determined.

It is interesting to observe that the percentage of nitrogen in the nucleo-albumin (16·14 p%) is much higher than in the mucins, which, according to the latest analyses, contain only from 11·7 to 12·3 p%.

In addition to the mucoid nucleo-albumin, bile is said to contain traces of normal mucin.

SECT. 12. THE CHOLESTERIN, FATS, SOAPS, LECITHIN AND REMAINING ORGANIC CONSTITUENTS OF THE NORMAL BILE.

In addition to its specific constituents—the salts of the bile acids and the bile colouring matters—and to the mucoid nucleo-albumin, the bile contains in solution the following organic substances:—cholesterin: palmitin, stearin and olein: alkaline salts (*i.e.* soaps) of palmitic, stearic and oleic acids: lecithin or its products of decomposition: a trace of urea: a trace of a diastatic ferment. All these bodies are described in other parts of this work, and the amounts in which they occur in the bile, so far as they can be determined, will be found in the tables exhibiting the results of the analyses of the bile in Sect. 15 of the present chapter. It is only, therefore, needful in this place to make a few remarks, chiefly with a view to emphasizing the relative importance of certain of these constituents.

The cholesterin[1] of bile. An absolutely constant constituent is cholesterin, which is present in the bile of man in the proportion of from 0·5—3·5 per 1000. This constituent, which is insoluble in water or aqueous saline solutions, is soluble in solutions of the salts of the bile acids and of soaps and neutral fats, and it is in virtue of these constituents that the bile is able to hold cholesterin in solution. When we inquire whether the cholesterin of the normal bile is formed in the liver or merely separated by it we come to the conclusion that, almost certainly, the liver, in reference to cholesterin, acts purely as an excretory organ. Cholesterin is one of the principal constituents of the white matter of the brain, of the spinal cord, and of medullated nerve fibres, and it is a constituent of the blood corpuscles; it appears most likely that the function of the liver, in reference to it, is to separate from the blood the excess of cholesterin which is set free in the metabolic changes which have their seat in the nerve centres.

This view rests upon theoretical considerations as well as upon certain facts, a thorough control of which is, however, urgently needed at the present time. It was for example asserted by Austin Flint, as a result of apparently careful investigations, that the blood of the jugular vein invariably contains much more cholesterin than that of the carotid artery. Although Flint based upon his researches a hypothesis which has not stood the test of further experiment, the facts above recorded have not hitherto been disproved. Again Frerichs and Becquerel and Rodier assert that in cases of jaundice, with complete obstruction to the flow of bile into the intestine, the amount of cholesterin in the blood is remarkably increased. It must be admitted that it is in the highest degree advisable that these particular determinations should be repeated, the accurate methods of analysis which we now employ being substituted for the cruder methods of the earlier enquirers.

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[1] The description of Cholesterin will be found in Vol. I. (1st edition), pp. 442—444.

Naunyn mainly on the ground of experiments on rabbits with temporary, and dogs with permanent biliary fistulæ, in which cholesterin was administered by the mouth or injected hypodermically, without sensibly increasing the amount of cholesterin excreted, comes (as the Author thinks) to the most improbable conclusion that the normal cholesterin of the bile is not derived from the blood but is of local origin, *i.e.* is derived from the mucous membrane of the gall-bladder. The crudeness, the obvious fallacies, attaching to the method of experimenting selected by Naunyn, are so obvious as to render any conclusions from his experiments much less trustworthy than those based on the observations of Frerichs. This distinguished physician cautiously asserted that whilst it is not certain, but only probable, that an increased quantity of cholesterin in the blood augments the amount of this constituent in the bile, the arrest of the excretion of the bile certainly leads to an increase of cholesterin in the blood[1].

There can be no question that cholesterin, besides being a constituent of the nervous tissues, the blood and the bile, exists in minute quantities in all animal and vegetable tissues rich in cells. It is present in small quantities in milk, but particularly it is produced in all pathological processes in which a destruction of cells occurs. It seems to be a product of the degeneration of cell protoplasm, especially where this is accompanied by the appearance of fatty matters; thus is explained its occurrence in pus. If we except milk, which is a secretion which is a product of specific transformations which occur in the protoplasm of the secretory cells of the mammary glands, and which lead to the formation of fat, no normal secretion but bile contains an appreciable quantity of cholesterin, and a process of reasoning by analogy would lead us to require evidence, such as has certainly not hitherto been adduced, before we could admit even the possibility of the cholesterin of *normal* bile being derived from the mucous membrane of the gall-bladder.

Lecithin.

The proportion of lecithin, or of the products of decomposition of protagen and lecithin in the bile, is much greater than in any other secretion of the economy—a fact which, taken in connection with its cholesterin excreting function, makes it not improbable that the liver is specially concerned in excreting certain metabolic products of the nerve centres.

Diastatic ferment.

In reference to the diastatic ferment, it is to be remarked that nearly all observations made on the bile of man, obtained from fistulæ, have revealed that the secretion possesses exceedingly feeble diastatic properties. No importance whatever can, however, be attached to the presence of such minute traces of ferment.

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[1] 'Ob die Zunahme der Cholesterinmenge im Blute während des höheren Alters einen steigenden Gehalt der Galle herbeiführe und darauf zum Theil die grössere Häufigkeit der Steine während dieser Lebensepoche beruhe, bleibt dahingestellt; es ist dies mehr als wahrscheinlich; jedenfalls influirt die Gallensecretion auf die Cholesterinmenge im Blut; sie nimmt zu, wo die Lebersecretion vermindert wird.' Frerichs, *Klinik d. Leberkrankheiten*, Bd. II. p. 486.

SECT. 13. THE MINERAL CONSTITUENTS OF NORMAL BILE.

In discussing, in the first volume of this work[1], the mineral constituents of the *liquor sanguinis* and *serum*, we have drawn attention to the difficulties which surround their investigation and have shewn how, in a measure, they have been overcome. In the case of the bile, these difficulties are so great (arising from the sulphur of the taurine and the phosphorus of the lecithin, which when ignited yield sulphuric and phosphoric acids, respectively) that the utmost reserve must be exercised in drawing conclusions, from the analyses of the ashes of the bile, as to the salts originally present in the secretion.

In addition to the organic sodium salts, the bile contains as its chief inorganic constituent sodium chloride, besides sodium carbonate and sodium phosphate. The quantity of potassium salts is, comparatively, very small. The ashes of bile contain, in addition, calcium phosphate, a trace of magnesium phosphate, a small but invariable quantity of iron, believed to exist as a phosphate (Hoppe-Seyler), and traces of copper; occasionally manganese has been likewise found. Although the published analyses of bile sometimes contain determinations of sulphates present in the ignited residue, it is probable that these salts are always the result of ignition; at most, the quantity of sulphates in the bile is exceedingly small.

The normal mineral constituents other than iron are best exhibited in the following analysis of the mineral matters of the bile of a healthy man made by Jacobsen[2].

Inorganic salts in 1000 parts, 8·5

KCl	0·28
NaCl	5·50
Na_3PO_4	1·30
Na_2CO_3	0·95
$Ca_3(PO_4)_2$	0·37
$FePO_4$	traces.

The Iron of the Bile.

When investigating for a Committee of the British Association the action of calomel and some other reputed cholagogues on the liver[3], Professor Rutherford and the Author were constantly struck by the reddish colour of the ashes of dog's bile, a colour due to the presence of oxide of iron.

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[1] Vol. I. (1st edition), p. 66—70.

[2] O. Jacobsen, *Ber. d. deutsch. chem. Gesell.* Vol. VI. p. 1026.

[3] 'Report on the Action of Mercury on the Biliary Secretion.' *British Association Reports*, 1868.

Gamgee and Young's observations.

At the suggestion and under the personal direction of the Author, Dr P. A. Young[1] subsequently carried out in the laboratory of the former, at Surgeons' Hall, Edinburgh, the first systematic investigation on the amount of iron in the bile of man, the dog and the ox.

Hoppe-Seyler and Kunkel's observations.

Subsequently, Hoppe-Seyler[2] and afterwards Kunkel[3] re-investigated the matter and amply confirmed the results obtained in the Author's laboratory. The following are the results obtained.

Quantity of Iron in the Bile.

I. Bile of Man	0·004 to 0·0105 p. cent. of Fe 0·0062 „	Young Hoppe-Seyler
II. Bile of Dog	0·016 p. cent. of Fe 0·0063 to 0·0078 „ „ 0·0036 to 0·0093 „ „	Young Hoppe-Seyler Kunkel
III. Bile of Ox	0·003 to 0·006 „ „	Young

The idea which suggested to the Author the research, which was carried out by Young, was that from the iron in the bile an estimate might be probably formed of the destruction of hæmoglobin occurring in the liver; there being strong grounds for assuming that bilirubin is formed by the decomposition of the hæmatin residue of hæmoglobin (see p. 349 *et seq.*). Kunkel found, however, that the relation of the iron to the bilirubin in the bile was as 1·4 or 1·5 : 100, whereas hæmatin contains 9 per cent. of Fe. It would therefore appear that, in the formation of bilirubin, there is a retention of iron in the liver, a fact which stands in close relation to the organic compounds of iron which Zaleski[4] shewed to be present in the liver. This subject will be further considered in Chapter V.

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[1] P. A. Young, M.D., 'On the relation which exists between the iron contained in the bile and the colouring matter of the blood.' *Journal of Anatomy and Physiology*, Vol. v. pp. 158—164; A. Gamgee, M.D., 'Note on Dr Young's paper.' *Ibid.* Vol. v. p. 165.

[2] Hoppe-Seyler, Analyses first published in his *Physiologische Chemie*, 2ᵉʳ Theil, Berlin, 1878, pp. 301 and 305.

[3] Kunkel, 'Eisen- und Farbstoffausscheidung in der Galle.' Pflüger's *Archiv*, Vol. xiv. (1877), p. 360.

[4] Zaleski, 'Studien über die Leber. I. Eisengehalt der Leber.' *Zeitschrift f. physiol. Chemie*, Vol. x. (1886), p. 453—502.

SECT. 14. THE GASES OF THE BILE.

The gases of the bile have been investigated by Pflüger[1], Bogoljubow[2], Noel[3], Charles[4] and, in so far as oxygen is concerned, by Hoppe-Seyler[5].

The bile is a secretion which is either entirely free from oxygen or contains it in very small proportions. As will be seen by referring to the subjoined tabular statement of the results obtained by Pflüger, he found in one analysis that 100 volumes of bile yielded 0·2 volumes of oxygen (measured at 0° C. and 1 M. pressure), whilst, in a second, oxygen was absent. Hoppe-Seyler investigated in the case of the bile, as in that of several other secretions, whether the liquid contained free oxygen, by allowing it to come in contact (under conditions which excluded the possibility of access of air) with reduced hæmoglobin. He found that, unlike the saliva, the bile contained no free oxygen or, to be more precise, that 100 volumes of bile must contain less than 0·15 volume of oxygen[6].

Excluding oxygen, which is either absent or present in very small quantities, the bile, when boiled in the mercurial pump, yields carbonic acid mixed with a small quantity of nitrogen. In addition to the carbonic acid, which is either free or so loosely combined as

VOLUME OF GASES (MEASURED AT 0° C. AND 1 M. PRESSURE) YIELDED BY 100 VOLUMES OF BILE FROM THE GALL-BLADDER OF DOGS (PFLÜGER).

	O	CO_2 (obtained by boiling in vacuo)	CO_2 (do. do. + acid)	N
I.	0·2	14·4	41·7	0·4
II.	0·0	5·0	0·6	0·6

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[1] Pflüger, *Archiv f. d. ges. Physiologie*, Vol. II. (1869), p. 156.

[2] Bogoljubow, *Centralblatt f. d. med. Wissenschaft*, 1869, no. 42.

[3] G. Noel, 'Étude générale sur les variations phys. des gaz du sang.' Thèse de Paris, 1876. Quoted by Hoppe-Seyler, *Phys. Chemie*, p. 306. The researches of Noel may be discarded as, from the amount of oxygen and especially of nitrogen which he found, it is certain that the gases which he obtained from the bile were mixed with large quantities of air which had leaked into his apparatus.

[4] J. J. Charles, 'Untersuchungen über die Gase der Lebergalle' (a. d. phys. Lab. Bonn). Pflüger's *Archiv*, Vol. XXVI. (1881), p. 200 *et seq.*

[5] Hoppe-Seyler, 'Ueber den Nachweis von absorbirtem Sauerstoffe in den Secreten mittelst Hämoglobin.' *Zeitschrift f. phys. Chemie*, Vol. I. (1877—78), p. 135.

[6] Hoppe-Seyler, *Physiologische Chemie*, p. 307.

to admit of being boiled out, the bile contains carbonic acid which can only be removed by the pump, after the addition of an acid. The total amount of carbonic acid as well as the relative amounts, free and combined, vary within surprisingly wide limits.

The analyses of Bogoljubow agree with those of Pflüger in demonstrating the remarkable and *as yet* inexplicable variations in the amount of CO_2 contained in bile as well as in the proportion which the free bears to the combined CO_2.

Charles, in the investigation which he made in Pflüger's laboratory, investigated the gases of the bile in rabbits, as well as in dogs. In the former animals, he found the volume of CO_2, especially of the CO_2 which could only be separated after the addition of phosphoric acid, to be very much larger than in dogs. From 100 volumes of the bile of these animals, he obtained from 102·37 to 114·9 volumes of CO_2 measured at 0° C. and 1 metre pressure. In one experiment, the CO_2 obtained by merely boiling in vacuo, only amounted to 9·75 volumes, whilst the combined CO_2 amounted to 105·18 volumes. In the case of a dog in a condition of deep narcosis 100 volumes of bile yielded 100·15 vols. of CO_2 (free and combined).

SECT. 15. SUMMARY OF THE QUANTITATIVE COMPOSITION OF THE BILE IN MAN AND CERTAIN OF THE LOWER ANIMALS.

Having now examined individually the various constituents of the bile, it appears desirable to group together the more important quantitative analyses which have been made of the bile of man and some of the lower animals.

Human Bile.

Total solids in the bile of biliary fistulæ and of bile collected in the gall-bladder.

The following table exhibits very clearly the difference in the proportion of solid matters in bile obtained from biliary fistulæ in the human subject and in bile obtained from the gall-bladder after death. The same difference is observable in the case of the lower animals. The old explanation that by remaining in the gall-bladder the bile loses water and becomes more concentrated is untenable and quite at variance with facts; the probable explanation is that when the secretion is cut off from the intestine and poured out externally, the liver secretes a bile which is relatively poor in solid matters (see p. 278 *et seq.*).

TABLE EXHIBITING DIFFERENCE IN PERCENTAGE OF SOLIDS IN FISTULA-BILE AS COMPARED WITH BLADDER-BILE.

Observers	Mean percentage of solids	Origin of bile
I[1].		
Jacobsen	2·26	Westphalen's case of thoracic biliary fistula. Male
Yeo and Herroun	1·35	Biliary fistula. Common bile duct occluded by cancerous nodule. Female
Copeman and Winston	1·42	Biliary fistula. Common bile duct occluded by gall-stone. Female
Mayo Robson and Fairley	1·81	Biliary fistula. Impacted gall-stones. Female
Noël Paton and J. M. Balfour	1·36	Biliary fistula. Common bile duct occluded by gall-stone. Female
II[2].		
Gorup-Besanez	13·96	Bile from gall-bladder of healthy individuals, executed, or dying by accident
Frerichs	14·04	

Complete analyses of fistula bile in the human subject.

In the following table, taken from the paper of Noël Paton and Balfour[3], are collected all the more recent analyses of fistula bile in the human subject. In Mr Mayo Robson's case, the analyses were made by Mr Fairley of Leeds.

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[1] All the cases under I. are referred to in the text, where references to the original observations will be found.

[2] The data under II. are taken from Gorup-Besanez, *Physiologische Chemie.* 4te Auflage, 1878, p. 519.

[3] D. Noël Paton, M.D., F.R.C.P. Ed., and John M. Balfour, M.B., C.M. 'On the Composition, Flow, and Physiological Action of the Bile in Man.' Vol. III. of the *Laboratory Reports* issued by the Royal College of Physicians. Edinburgh, 1891. See p. 203.

	Jacobsen	Yeo and Herroun	Copeman and Winston	Mayo Robson	Paton and Balfour	
					Sept. 1	Sept. 7
Cholesterin	0·056	0·038		0·045	0·053	0·075
Lecithin	0·005		0·099	—		
Fats	0·01			0·012	0·009	
Glycocholate of Soda	1·01	0·165	0·628	0·751	0·356*	0·349
Taurocholate of Soda	—	0·055		0·009	0·049*	
Soaps	0·14	—	—	0·097	0·015*	—
Mucin, Pigments, Epithelium, &c.	0·23	0·148	0·1725	0·130	0·7096	0·461
Inorganic	0·85	0·840	0·451	0·758		0·641
Chlorides	0·578	0·716	—	0·501		
Solids	2·26	1·284	1·423	1·802	1·1919	1·527
Water	97·74	98·716	98·577	98·198	98·8080	98·479

* The acids are here given.

In these analyses the fact is brought out very distinctly that, in the bile of man, glycocholic acid preponderates very greatly over taurocholic acid, which may even be altogether absent (see Jacobsen's analysis). The same fact results from the analyses made, by the most competent observers, in the case of bladder bile.

Complete analyses of bladder-bile in the human subject.

The following exhibit the method of analyses of 5 samples of human bile (taken *post mortem* from the gall-bladder) made by Hoppe-Seyler in 1872[1].

Mucin in 100 parts	1·29
Other organic matters insoluble in alcohol	0·14
Sodium taurocholate	0·87
Sodium glykocholate	3·03
Soaps	1·39
Cholesterin	0·35
Lecithin	0·53
Fats	0·73
Phosphate of iron	0·0166

Ratio of sodium glykocholate to sodium taurocholate is as 3·4 : 1.

Bile of the Dog.

The following analyses made by Hoppe-Seyler[2] illustrate the composition of the bile of the dog. In this animal the bile *never* contains glykocholic acid.

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[1] Published for the first time in his *Physiologische Chemie*, 2ter Theil, Berlin, 1878, p. 301.

[2] *Physiologische Chemie*, p. 302.

Constituents	Bladder-bile		Bile from fistula	
	I.	II.	I.	II.
Mucin in 100 parts	0·454	0·245	0·053	0·170
Alkaline taurocholate	11·959	12·602	3·460	3·402
Cholesterin	0·449	0·133	0·074	0·049
Lecithin	2·692	0·930	0·118	0·121
Fat	2·841	0·083	0·335	0·239
Soaps	3·155	0·104	0·127	0·110
Other organic matters insoluble in alcohol	0·973	0·274	0·442	0·543
Inorganic matters insoluble in alcohol	0·199	—	0·408	—
K_2SO_4	0·004	—	00·22	—
Na_2SO_4	0·050	—	00·46	—
$NaCl$	0·015	—	0·185	—
Na_2CO_3	0·005	—	0·056	—
$Ca_3(PO_4)_2$	0·080	—	0·039	—
$FePO_4$	0·017	—	0·021	—
$CaCO_3$	0·019	—	0·030	—
MgO	0·009	—	0·009	—

Bile of certain other animals.

The following table[1] exhibits the results of old analyses of the bile of the gall-bladder of various animals.

Constituents	Ox[2]	Pig[3]	Kangaroo[4]	Goose		Python[7]
				I[5]	II[6]	
Mucin and pigment	0·30	0·59	4·34	2·56	3·1	0·89
Bile-salts	8·00	8·38	7·59	14·96	16·4	8·46
Cholesterin, lecithin, and fat		2·23	1·09	0·36	0·3	0·03
Inorganic salts	1·26			2·10	2·6	0·20
Total solids	9·56	11·20	44·13	19·98	22·4	9·58
Water	90·44	88·80	85·87	80·02	77·6	90·42

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[1] Halliburton's *Text-Book of Chemical Physiology*, London, 1891, p. 678.

[2] Berzelius, *Lehrbuch*, Dresden, 1831.

[3] Gundlach und Strecker, *Ann. Chem. Pharm.* LXII. 205.

[4] Schlossberger, *Ibid.* CX. 244.

[5] Marsson, *Ann. d. Pharm.* LVIII. 138.

[6] Otto, *Ann. Chem. Pharm.* CLIX. 189.

[7] Vogtenberger und Schlossberger, *Ibid.* CVIII. 66.

CHAPTER V.

THE BILE (*continued*).

SECT. 1. RECAPITULATION OF THE FACTS RELATING TO THE ORIGIN OF THE SPECIFIC CONSTITUENTS OF THE BILE.

IN the preceding chapter, the subject of this section has been more than once referred to. It appears advisable, however, in this place, briefly, but systematically, to refer to the different facts which throw light on the probable antecedents of the specific constituents of the bile.

The origin of the bile acids.

The glycocine and taurine of the conjugated bile acids are, without doubt, derived from the albuminous or albuminoid principles of the economy. Glycocine we have seen to be one of the products of the decomposition of gelatine, when this body is subjected to long boiling with acids, or to the digestive action of trypsin. Taurine, although it has not been artificially obtained by the decomposition of proteids is, as its high percentage (14·6 p%) of sulphur indicates, certainly derived from them in the body. The taurine and the glycocine of the bile, however, only contain a small fraction of the sulphur and of the nitrogen corresponding to the decomposition of the proteids in the economy. "If the bile were an excretion like urine, we should expect to find the quantity of nitrogen and sulphur in the bile varying proportionally with the amount of proteid decomposed in the body. As a matter of fact, this is not the case. We know from the researches of Kunkel[1] and Spiro[2], conducted on dogs with biliary fistulæ, that only a small part of the sulphur and nitrogen resulting from proteid metabolism appears in the bile, and that it is but very slightly increased by a larger supply of food. When the amount of albumin allowed the dog was multiplied eightfold, the nitrogen and sulphur of the bile were only doubled[3]."

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[1] Kunkel, 'Untersuchungen über den Stoffwechsel in der Leber.' Pflüger's *Archiv*, Vol. XIV. (1876), p. 344.

[2] Spiro, 'Ueber die Gallenbildung beim Hunde.' Du Bois Reymond's *Archiv*, 1880. Suppl. p. 50.

[3] Bunge, *Physiological and Pathological Chemistry*. Woolridge's Translation, Vol. I. p. 214.

The origin of the bile colouring matters. As soon as the remarkable resemblance, if not the absolute identity, of Virchow's hæmatoidin with bilirubin was pointed out, the clue to the origin of the bile colouring matters was discovered.

"In extravasations of blood, the colouring matter of the blood disappears and in place of it we find a crystallised pigment, which Virchow[1] was the first to examine carefully and named hæmatoidin. The same writer pointed out its resemblance to bile pigment[2]. Subsequently Robin[3], Jaffé[4] and Salkowski[5] proved the identity of hæmatoidin and biliverdin. Langhans[6] took the blood from the vein of a living pigeon and injected it under the skin of the same animal; after two or three days the colouring matter of the blood had disappeared from the subcutaneous clot and was replaced by bilirubin and biliverdin. Quincke[7] performed the same experiment on dogs. In this case the conversion occupied more time: the bilirubin did not appear in the subcutaneous injection before the ninth day. Cordua[8] injected blood into the abdominal cavity of dogs and found bilirubin after so short a time as thirty-six hours. Finally Recklinghausen[9] has seen bile pigment formed in the blood of frogs outside the body, after from three to ten days[10]." The observations here recorded have been confirmed and extended by the experiments of Latschenberger[11] who injecting the blood corpuscles of the horse, or a magma of hæmoglobin prepared from horse's blood into the subcutaneous areolar tissue of another horse found, after some days, reddish yellow pigments (*Choleglobine*) at the seat of injection, and in addition dark pigmentary matter containing iron (1) which he designated '*Melanin*'; Neumann[12] had previously found a similar pigment in blood extravasations and thrombi in man and had designated it *hæmosiderin*. According to Kunkel[13], the latter consists of ferric hydrate.

A study of the empirical formulæ of hæmatin and of bilirubin shewed[14] how closely bilirubin resembled in composition an iron free hæmatin. According to Nencki and Sieber[15], the following equation probably expresses the transformation of hæmatin into bilirubin

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[1] Virchow's *Archiv*, Vol. I. (1847), pp. 379, 407.
[2] Virchow, *loc. cit.* p. 445.
[3] Robin, *Comptes Rendus*, Vol. XLI. (1855), p. 506.
[4] Jaffé, Virchow's *Archiv*, Vol. XXIII. (1862), p. 192.
[5] Salkowski, Hoppe-Seyler's *Med. chem. Unters.* Heft III. (1868), p. 436.
[6] Langhans, Virchow's *Archiv*, Vol. XLIX. (1870), p. 66.
[7] H. Quincke, Virchow's *Archiv*, Vol. XCV. (1884), p. 125.
[8] Herm. Cordua, 'Ueber den Resorptionsmechanismus von Blutergüssen.' Berlin, Hirschwald, 1877.
[9] Recklinghausen, *Handbuch d. allgem. Path. d. Kreislaufes und der Ernährung*, p. 434. Stuttgart, Enke, 1883.
[10] Bunge, *Op. cit.*, p. 376.
[11] Latschenberger, 'Die Bildung des Gallenfarbstoffes aus dem Blutfarbstoff.' *Monatschrift f. Chemie*, Vol. IX. (1888), p. 52. Quoted by Neumeister, *Lehrbuch der physiologischen Chemie*, p. 174.
[12] Neumann, 'Beiträge zur Kenntniss der pathologischen Pigmente.' Virchow's *Archiv*, Vol. CXI. (1888), p. 25.
[13] Kunkel, 'Ueber das Vorkommen von Eisen nach Blutextravasation.' *Zeitschrift f. phys. Chemie*, Vol. V. (1881), p. 40.
[14] Kühne, *Lehrbuch*, p. 203.
[15] Nencki und Sieber, 'Untersuchungen über den Blutfarbstoff.' *Ber. d. deutsch. chem. Gesellschaft*, Vol. XVII. (1884), p. 2275.

$$\underbrace{C_{32}H_{32}N_4O_4Fe}_{\text{Hæmatin}} + 2H_2O - Fe = \underbrace{C_{32}H_{36}N_4O_6}_{\text{Bilirubin}}$$

The iron free hæmatoporphyrin, which, according to Nencki and Sieber, has the same percentage composition as bilirubin, and which is represented by the formula $C_{16}H_{18}N_2O_3$, when reduced by means of tin and hydrochloric acid in alcholic solution soon yields a body which confers a yellow colour to the solution, and which in its behaviour towards solvents, and in its spectrum, is undistinguishable from hydrobilirubin, which we have seen to be the typical product of the action of nascent hydrogen on bilirubin (Hoppe-Seyler[1]).

In addition to the above facts, which alone are perhaps sufficient to prove that the bile colouring matters are derived from the hæmatin of hæmoglobin, there are others which tend in the same direction. Thus *Amphioxus* which is the only vertebrate without red blood corpuscles forms no bile colouring matters (Hoppe-Seyler[2]).

Again, it has been shewn that, concurrently with the excretion of the bile colouring matters, the liver always excretes iron, though the amount of the latter is not in proportion to the former, assuming the decomposition to go on, as expressed in the equation of Nencki and Sieber.

That all the iron, resulting from the decomposition of hæmatin which leads to the formation of bile colouring matters, should not be excreted in the bile is, however, in no way surprising. The fact, indeed, agrees with others with which we are acquainted. It has already been stated that Zaleski found various combinations of iron in the liver. In some of these the metal can be detected by the reagents usually employed for the detection of iron in its salts (*e.g.* by potassium ferrocyanide and hydrochloric acid), but in others the metal is an element of organic compounds, such as nucleins, and can only be detected when these are destroyed. In one form or another, the liver appears to hoard a certain proportion of iron which probably takes part in fresh syntheses of hæmoglobin. When, however, the destruction of red blood corpuscles goes on with abnormal rapidity, as appears to be the case in *pernicious anæmia* (Hunter[3], Mott[4], Delépine[5], and in poisoning by arseniuretted hydrogen (Naunyn and Minkowski[6]), the iron in the liver increases greatly. In the first case, the urine contains abnormal quantities of urobilinoid matters, which are certainly close derivations of the bile colouring matters; in the second case much bilirubin.

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[1] Hoppe-Seyler, *Physiologische Chemie*, p. 398.

[2] Hoppe-Seyler, Pflüger's *Archiv*, Vol. XIV. (1877), p. 399; *Physiologische Chemie*, p. 276.

[3] Hunter, *Lancet*, 1888, Vol. II. pp. 555, 608, 654.

[4] Mott, *Lancet*, 1889, Vol. I. p. 520; 1890, Vol. I. p. 287; *Practitioner*, Aug. 1890.

[5] Delépine, *Practitioner*, Aug. 1890.

[6] Minkowski und Naunyn, *Archiv f. exp. Pathol. und Pharmakol.* Vol. XXI. (1886), p. 1.

It has thus been proved, by a number of distinct but correlated arguments, that the bilirubin of the bile is a derivative of the blood colouring matter. When discussing the mode of production of jaundice, we shall shew that, if we except the local origin in blood extravasations, bilirubin is only formed in the liver.

SECT. 2. DISCUSSION OF THE QUESTION WHETHER THE BILE IS TO BE CONSIDERED A DIGESTIVE SECRETION. THE ACTION OF THE BILE ON CARBOHYDRATES, PROTEIDS AND FATS.

Preliminary Observations.

The bile differs from the saliva, the gastric juice, and the pancreatic secretion in that, if we except altogether insignificant traces of a diastatic enzyme, it contains no ferment capable of dissolving, or effecting the hydrolytic decomposition of, carbohydrates, proteids, or fats. Nevertheless, although the bile unquestionably subserves excretory functions, it also plays an auxiliary and not unimportant, part in the complex digestive processes of the small intestine.

Two interesting anatomical considerations may be adduced, of which the first establishes the excretory function of the bile, whilst the second raises a strong presumption in favour of the importance of the bile in the digestive process:—

Firstly. During the third month of fœtal life[1], long before other digestive secretions are found out (at a time therefore when digestive acts are yet entirely in abeyance), the liver whose importance for the economy at this period is, doubtless, represented by its relatively enormous development, commences to secrete bile which accumulates in the intestine and forms a large part of the *meconium*[2], which is discharged at an after birth. This fact argues strongly in favour of the indispensable excretory functions of the bile.

Secondly. The bile duct in all animals opens into the commencement of the intestine, into the duodenum, either at the same place as, or above the, principal pancreatic duct. 'If,' argues Bunge[3], 'the bile were an excretion we should expect the ductus choledochus to open into the lower end of the rectum, just as the ureter opens into the cloaca in the lower vertebrates. It is impossible not to believe that bile, in its long passage through the intestines, must have some serious duties to perform.'

[1] Zweifel, 'Untersuchungen über den Verdauungsapparat des Neugeborenen,' Berlin, 1874, *Centralblatt f. d. med. Wissenschaft*, 1874, no. 59; Preyer, *Specielle Physiologie des Embryo*, Leipzig, 1885.

[2] Zweifel, 'Untersuchungen über das Meconium.' *Archiv f. Gynäkologie*, Vol. VII. p. 474.

[3] Bunge, *Physiological and Pathological Chemistry*, Vol. I. p. 213.

With Bunge, we consider, as already stated, that this anatomical fact establishes a strong presumption in favour of the view that, in its long passage through the intestine, the bile must have serious duties to perform, though we cannot admit that it negatives the view that the bile is in respect of certain constituents (cholesterin and bile colouring matters) *an excretion*. Altogether independent of such arguments, however, we are in possession of facts which establish in the clearest manner that the bile, besides being an agent in the removal of waste products from the economy, is essential to the *normal course* of the digestive processes of the small intestine: that without its aid a most important group of alimentary principles—the fats—in great part invariably escape absorption, though, it is true, this departure from the normal state may, in some cases, be not inconsistent with the persistence of an apparently satisfactory state of the general health.

Action of bile on starch. Many observers have shewn that the bile obtained from human biliary fistulæ, if digested with starch solutions, for a great many hours, is able to convert traces of starch into sugar. It is obvious, however, that in virtue of these traces the bile can play no part in the digestion of starches, and accordingly it has been found[1] that the fæces of dogs with biliary fistulæ and fed upon bread either contain no starch or only such small quantities as occur in the case of normal dogs fed in the same manner. These experiments are borne out by observations made on human subjects with biliary fistulæ.

The bile of the pig appears, according to the observations of Nasse[2], to possess the power of dissolving raw starch.

Action of bile on proteids. The bile, containing no proteolytic enzyme, exerts no *direct* action in digesting the albuminous and albuminoid matters. Nevertheless, the bile must *indirectly* co-operate in the digestion of proteids. When bile is added to the acid products of gastric digestion (chyme), a precipitate is produced[3], composed essentially of a mixture of unchanged native albumins, and of bile acids (especially taurocholic acid), which carries down with it pepsin (Brücke). The important result of the admixture is, however, the fact that, though the quantity of bile may be quite insufficient to neutralise the acidity of the chyme, the proteolytic activity of the pepsin is at once arrested (Brücke). The bile seems to have, therefore, the important function of arresting peptic digestion, and in this way, as well as in others, of establishing the conditions which are requisite for the proper exercise of the digestive activity of the pancreatic juice.

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[1] Bidder und Schmidt, *Verdauungssäfte*, p. 222.

[2] H. Nasse, Canstatt's *Jahresbericht d. Pharm.* 1859, II. S. 33 (quoted by Maly, Hermann's *Handbuch*, Vol. VII. p. 177.

[3] Bernard, *Leçons de Physiologie Expérimentale*, Paris, 1856, p. 422; Kühne, *Lehrbuch d. phys. Chemie*, p. 99; J. Moleschott, 'Ueber die Einwirkung der Galle und ihrer wichtigsten Bestandtheile auf Peptone.' *Untersuchung zur Naturlehre der Menschen*, Bd. XI. Heft 5, s. 2.

Our knowledge of the reactions which occur when the bile comes in contact with an acid chyme is derived mainly from the writings of Kühne[1] and of Hammarsten[2] and from the more recent researches of Maly and Emich[3]. The principal results will here be summarised. If bile, which has been freed from its mucoid nucleo-albumin by treatment with alcohol, be added to a mixture made by digesting egg albumin in artificial gastric juice, a precipitate occurs, which is composed partly of a heavy flocculent body and partly of a finely granular body which it is hard to separate by filtration. The former is a compound of a proteid with bile acids; the latter is composed essentially of bile acids mixed, perhaps, with a small quantity of albumoses. The flocculent precipitate to which we have referred, and which was formerly spoken of as containing syntonin, doubtless does not usually contain acid albumin, but the body which Meissner termed *Parapeptone*, and Kühne *Antialbumat* (see pp. 115 and 120).

The amount of the two precipitates produced depends greatly upon the acidity of the digestive mixture, upon the composition of the bile which is added and upon the quantity of salts which may be present. There are, for instance, specimens of ox bile which are precipitable when treated with dilute acids, and others which are not; the former contain relatively very little taurocholic acid, the latter much more. When the former are mixed with bile at the temperature of the body, a precipitate is thrown down; if the liquid be filtered and cooled, a fresh precipitate forms which is sometimes so abundant as to give to the liquid the consistence of a magma. When the bile is rich in taurocholic acid this precipitation does not occur, or the precipitate at first produced is readily dissolved on adding more bile (see p. 296).

Although, as a result of Hammarsten's researches, it was shewn that the precipitate produced by adding bile to acid chyme was partly proteid in nature and partly composed of bile acids, accurate information was wanting as to the exact power which the two principal bile acids possess of precipitating:—firstly, native albumins: secondly, albumoses: thirdly, peptones. Experimenting with solutions of the pure bile acids or their salts, and mixing with them solutions of native albumins, and of albumoses and of peptones as pure as it was at that time possible to obtain them, Maly and Emich obtained the following interesting results:—

First. Taurocholic acid (or a mixture of an alkaline taurocholate and an acid) precipitates native albumin or acid albumin at least as completely as do tannic or phospho-tungstic acids. On the other hand, taurocholic acid preciptates neither albumoses nor peptones.

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[1] Kühne, *Lehrbuch*, pp. 98—100.

[2] Hammarsten, Abstract in *Jahresber. d. ges. Med.* 1870, Vol. I. p. 106. See also Maly in Hermann's *Handbuch*, Vol. V. II. 181.

[3] Maly und Emich, 'Ueber das Verhalten der Gallensäuren zu Eiweiss und Peptonen und über deren antiseptische Wirkungen.' *Monatschrift f. Chem.* Vol. IV. (1883), pp. 89—120.

Second. The precipitate which occurs when taurocholic acid is mixed with a solution of albumoses or peptones is composed of taurocholic acid.

Third. Glykocholic acid neither precipitates native albumins nor albumoses and peptones. When crystallising from a solution, glykocholic acid may, however, carry down with it mechanically traces of albumoses. Further, glykocholic acid, unlike taurocholic acid, is not precipitated by albumoses or peptones.

The whole of the facts which we have endeavoured to bring together establish that when the addition of bile, which contains both glykocholic and taurocholic acid, to chyme, leads to a precipitate, this may contain, firstly a precipitate of parapeptone or antialbumat, thrown down by taurocholic acid: secondly, a precipitate of glykocholic acid, due to the mere acidity of the mixture, if the quantity of taurocholic acid in the bile was very small: thirdly, a precipitate of taurocholic acid. Both the second and third of these are very readily soluble in faintly alkaline solutions, and excess of bile, therefore, readily dissolves the precipitate which at first occurred.

It follows from the researches of which an account has been given that bile which contains taurocholic acid, typically that of the carnivora, can effect a very perfect precipitation of native albumin or of syntonin, if any should exist in solution and what is much more important of the parapeptone, which is incapable of further action by pepsin, and which always is present in greater or less quantity, however long the process of gastric digestion has gone on. That such a precipitation is actually to be observed in the duodenum, firmly adhering between the villi, is asserted by so infallible an observer as Kühne[1], who, however, states that a little further down the precipitate is no longer observed. This precipitation has been supposed to favour the action of the pancreatic juice upon the albuminous bodies which have escaped conversion into albumoses, for it is only the former, as we have shewn, which are precipitated.

It appears to the Author that the precipitation of the chyme by the bile, which chiefly leads to the temporary separation of the bile acids, may be of special importance in placing the latter under conditions which will favour their decomposition and rapid reabsorption. All facts seem to give support to the view first advocated by Schiff (see p. 278 *et seq.*) and to shew that very soon after the bile has been deflected from its normal course into the alimentary canal, the solid matters which it contains and which consist principally of the salts of the bile acids, undergo a great diminution : whilst very soon after the bile has been allowed to resume its normal course the bile acids in the bile increase. May not then the absorption of the bile and, perhaps, the decomposition into cholalic acid and amido-acids which probably precedes absorption, be a function which is assisted by the precipitation which we have been considering?

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[1] Kühne, *Lehrbuch*, p. 99.

The Action of the Bile on Fats.

It has already been stated that the digestion of starchy and proteid constituents of food proceeds normally in animals or human beings with biliary fistulæ, the fæces containing no larger quantity of undigested starches or proteids than in the normal condition of the body. The case is, however, very different with the fats. As Bidder and Schmidt first ascertained with precision, and as has been abundantly and invariably confirmed, whenever the bile is cut off from the alimentary canal, the fæces contain a large quantity of fat, shewing that the processes which are necessary precursors of its absorption have been materially interfered with.

Bidder and Schmidt found that dogs with biliary fistulæ absorbed from 2½ to 7 times less fat than normal dogs. They also found that whilst dogs on a meat diet yielded a milk-white chyle which contained 3·2 per cent. of fat, in dogs in which biliary fistulæ had been long established the chyle, instead of being milk-white, merely presented an opalescent appearance and, in one case, contained only 0·19 per cent. of fat. Voit[1] shewed that when dogs are fed on quantities of fat amounting to from 150 to 200 grms., 99 per cent. of the total amount is absorbed, only about 1 per cent. passing into the fæces, whilst if the same amount of fat be given to dogs with biliary fistulæ, as much as 66 per cent. of the whole amount ingested is excreted *per anum.* It is to this incapacity of utilizing the fats that Voit ascribes the emaciation, as well as the ravenous hunger, of many dogs with biliary fistula.

The analyses of the fæces of human beings with biliary fistulæ have shewn that these invariably contain much fat; usually from 11 to 13 per cent. of their weight. This fact will be referred to again.

In describing the properties of the bile, we have referred to the fact that it is able to dissolve small quantities of neutral fats, so that it has, from time immemorial, been employed to remove grease stains from coloured fabrics. That this slight solvent action of the bile on the neutral fats plays a material part in the processes of the intestine is, however, most unlikely. We have also referred to the fact that the bile, when shaken with liquid fats, forms imperfect emulsions, from which the oil soon separates.

Although bile, pure and unmixed, possesses this imperfect emulsionising power, the case is very different if it be mixed at the temperature of the body with free fatty acids. Under these circumstances, an acid emulsion is obtained, holding an excess of fatty acids in solution and capable of forming a perfect emulsion with the neutral fats. The process is one in which soaps are produced through the decomposition of the alkaline salts of the bile acids by the free fatty acids. As a matter of fact, the bile in the intestine finds itself in contact with fatty acids which have been liberated from the neutral fats by the fat-decomposing enzyme of the pancreatic juice, and all the conditions are present which are requisite to confer on the bile an emulsionising action. How far this action is important in the mechanism of the absorption of fats will be further considered when that subject is being treated of in detail.

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[1] Carl Voit, 'Ueber die Bedeutung der Galle für die Aufnahme der Nahrungsstoffe im Darmkanal.' Stuttgart. Verlag der J. G. Cotta'schen Buchhandlung, 1882. (Separatabdruck aus den '*Beiträgen der Biologie, Jubiläumsschrift für Geheimrath v. Bischoff.*')

But the action of the bile on the fats is not limited to the formation of soaps with the free fatty acids Neumeister[1] points out that solutions of the cholates possess the power, when gently heated, of dissolving the insoluble soaps of calcium and magnesium, compounds which doubtless occur in the intestine. In considering the absorption of fats, we shall also discuss the supposed influence of the bile in aiding the passage of fats through membranes.

The Antiseptic and Laxative Actions of the Bile.

It is impossible to make observations on dogs with biliary fistulæ without being struck by the peculiar fœtor of the fæces, which reminds one of the fœtid smell emanating from vats in which skeletons are macerating. The clay-coloured fæces of human beings with biliary fistulæ, or suffering from jaundice depending on a complete obstruction to the flow of bile in the intestine, almost invariably present somewhat of the same peculiar fœtor. These facts naturally led physiologists (Bidder and Schmidt) and physicians to ascribe to the bile an antiseptic action on the contents of the intestine. That the bile is in any sense an antiseptic has, however, been denied by some, inasmuch as it is a liquid which when exposed to air rapidly decomposes; the experiments of Sherrington[2] and of Copeman and Winston[3] have shewn, moreover, that microorganisms, of the most diverse kinds, develope perfectly in culture media to which bile has been added.

It has already been said that the clay-coloured fæces of animals with biliary fistulæ, as well as of human subjects with obstruction of the common bile-duct, when fed upon a mixed diet, contain undigested fat. It is, indeed, this unabsorbed fat, which amounts to 11 or 13 per cent. of the weight of the fæces, which is the chief cause of the clay-coloured appearance which they present. It has been found, that when an animal is fed upon a diet in which flesh is absent the putrid decomposition which causes the peculiar fœtor no longer occurs[1, 2]. It has, of late, been maintained that it is not the withdrawal of the antiseptic action of the bile which is the cause of this putrid decomposition but the presence of unabsorbed fat in the contents of the large intestine. The fat is supposed to act by preventing the proper digestion of the albuminous food substances, which, being enveloped in fat, undergo putrefaction. It may be true that the presence of fats favours somewhat the putrefactive decomposition of the proteids, though it appears to the Author that it is quite inadequate to explain it. The fæces of children as well as of adults when living upon a diet of milk alone contain far larger quantities of undigested proteids than the fæces of animals with biliary fistulæ, and they contain likewise much undigested fat, and yet there is usually a remarkable absence of fœtor and particularly of that which characterises the fæces in which no bile is present. The same remark applies to cases in which large quantities of vegetable or animal oils are administered and in which the fæces always contain much fatty matter which has escaped decomposition. In two cases of fatty stools associated with disease of the pancreas, without pressure on the common bile-duct, which the Author has had occasion to observe, there was, in spite of large quantities of unabsorbed fat, a complete absence of the fœtor under discussion. How then can we explain the disappearance of the fœtid decomposition when dogs with biliary fistulæ are fed on a diet in which fats have been replaced by carbohydrates? Doubtless, the acids resulting from the fermentation of carbohydrates in the intestines exert an influence on the alimentary contents which adequately compensates for that normally exerted by the bile acids.

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[1] Neumeister, *Lehrbuch d. phys. Chemie*, 1893, p. 178.

[2] C. J. Sherrington, *Journal of Pathology and Bacteriology*, Feb. 1893.

[3] Copeman and Winston, *op. cit.*, *Journ. of Physiology*, Vol. x. (1889), p. 226. On this matter compare the experiments of Limbourg, *Zeitschrift f. phys. Chemie*, Vol. XIII. p. 196.

Maly and Emich[3] have shewn that the free bile acids, and especially taurocholic acid, exert a powerful antiseptic action, and their statements have been confirmed by the observations of Linderberger[4]. Admitting that free taurocholic acid is nearly as powerful an antiseptic as salicylic acid, as Maly and Emich assert, it may be urged that, as the bile does not contain free bile acids, any argument based on the properties of these is faulty. The reader is reminded, however, that the matter is not as simple as it appears at first sight to be. In the first place, we have seen that in three various ways we may have the bile acids precipitated when the bile comes in contact with the acid chyme: viz. in combination with syntonin or with antialbumat if these substances are present and the bile contains

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[1] Voit, *op. cit.*

[2] F. Rohmann, 'Beobachtungen an Hunden mit Gallenfisteln,' Pflüger's *Archiv*, Vol. XXIX. (1883), p. 295.

[3] Maly und Emich, 'Ueber das Verhalten der Gallensäuren zu Eiweiss und Peptonen und über deren antiseptische Wirkungen,' *Monatschrift f. Chemie*, Vol. IV. (1883), pp. 89—120.

[4] V. Linderberger, 'Ueber die Bedeutung der Galle für die Fäulnissprocesse im Dünndarm.' Abstracted from the original Swedish paper, by Hammarsten. Maly's *Jahresb.* Vol. XIV. (1885), p. 334.

taurocholic acid in a free condition; as a result of the acidity of the chyme, if glykocholic acid preponderates greatly; in a free condition by the action of the albumoses and peptones existing in solution, if taurocholic acid be present. But leaving the chyme altogether out of consideration, it is perfectly certain that the salts of the bile acids must be decomposed by the intestinal contents in virtue of the free acids which they invariably contain. As Nencki has pointed out, the contents of the small intestine are invariably acid, in consequence of the development of organic acids, and especially of lactic acid, through the agency of micro-organisms on the sugars. It does not appear to be a very bold assumption to suppose that this precipitation of the bile acids modifies, in an important manner, the subsequent changes which the albuminous bodies undergo in the intestine, under the influence of putrefactive organisms; it is, nevertheless, obvious that this restraining action cooperates with and may be replaced by other agents.

In addition to its other functions, the bile is supposed to exert a naturally laxative action, inasmuch as, when it is cut off from the intestine, constipation is a frequent result, whilst when it is administered medicinally, in the form of *fel bovinum inspissatum*, it exerts a laxative action, or reinforces the action of drugs possessing a laxative action[1].

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[1] Refer to facts referring to this matter in Brunton's *Pharmacology, Therapeutics and Materia Medica.* London, 1887, see p. 1082.

CHAPTER VI.

THE MODE OF PRODUCTION AND THE PHENOMENA OF ICTERUS, OR JAUNDICE. ICTEROGENIC POISONOUS AGENTS. THE MODIFICATIONS IN CHEMICAL COMPOSITION WHICH THE BILE EXHIBITS IN DISEASE. THE INFLUENCE OF DRUGS ON THE SECRETION OF BILE—CHOLAGOGUES. THE ELIMINATION OF MEDICINAL AND POISONOUS AGENTS BY THE BILE.

SECT. 1. THE MODE OF PRODUCTION AND THE PHENOMENA OF ICTERUS OR JAUNDICE. ICTEROGENIC POISONOUS AGENTS.

JAUNDICE is a condition characterised by a more or less deep yellow, or greenish-yellow, colour of the skin and conjunctivæ and by the presence of bile colouring matters in the urine. It invariably depends upon causes which either completely obstruct the flow of bile, or which modify the pressure under which the bile normally flows along the biliary ducts.

When, from any cause, the pressure increases in the bile ducts, the bile colouring matter and the bile acids enter the lymphatics of the liver and are conveyed in the lymph through the thoracic duct into the blood (v. Fleischl[1], Kunkel[2]). If the thoracic duct, as well as the common bile duct, be ligatured, no jaundice results and biliary constituents cannot be detected in the urine (Kufferath[3]). Vaughan Harley[4] in a recent research, conducted under Ludwig's direction, has confirmed in a remarkable manner the results of v. Fleischl, Kunkel and Kufferath. Having ligatured the common bile duct and the thoracic duct, he found that dogs often survive the operation, without any appreciable inconvenience and without jaundice supervening; neither bilirubin nor bile acids passed into the urine, though in one case seventeen days elapsed between the date of the application of the ligatures and that on which the animal was killed. It, therefore, appears to be proved, beyond the possibility of doubt, that it is only through lymphatic paths that the biliary constituents can leave the liver and enter the blood. The bile colouring matter first makes its appearance in the urine, and if the quantity entering the blood cannot *pari passu* be excreted by the kidneys, it is deposited in, and stains, in the first instance, the conjunctivæ, afterwards the skin and other tissues.

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[1] v. Fleischl, 'Von der Lymph und den Lymphgefässen der Leber.' Ludwig's *Arbeiten*, 1874, p. 24.

[2] Kunkel, Ludwig's *Arbeiten*, 1875.

[3] Kufferath, 'Ueber die Abwesenheit der Gallensäuren im Blute nach dem Verschluss des Gallen- und des Milchbrustganges' (Aus d. phys. Inst. zu Leipzig). Du Bois Reymond's *Archiv*, 1880, pp. 92—94.

[4] Dr Vaughan Harley, 'Leber und Galle während dauernden Verschlusses von Gallenund Brustgang' (Aus d. phys. Inst. zu Leipzig). Du Bois Reymond's *Archiv*, 25th May, 1893, p. 291 *et seq.*

Although the specific constituents of the bile cannot, in the normal condition, be detected in the blood, there can be little doubt that the fact is due not to their complete absence (seeing that one and probably both find their way normally, in minute quantities, into the lymph of the thoracic duct) but to their amount being too small to admit of detection.

Tappeiner[1], though unable to detect bile acids in the blood of dogs, was able to separate them from the lymph of the thoracic duct and to identify them. Hammarsten (see p. 315) has shewn that bilirubin is a normal constituent of horse's blood. Unless the quantity either of bile acids or bile colouring matters becomes too large, they are, however, excreted by the liver and do not occur, even in traces, in the urine.

Having, in the above sentences, briefly summarized the results of modern inquiry, as to the mode of production of jaundice, it is necessary to refer to, and to discuss in some detail, the grounds upon which certain views, which until lately obtained very general support, have fallen into discredit, or rather have been proved to be false.

Until comparatively recent times, knowledge as to the mode of origin of the bile colouring matter and bile acids was so wanting in precision that, as a necessary consequence, the mechanism of jaundice was entirely misunderstood. By a great majority of physiologists and physicians, notably by our own Glisson[2], the liver was looked upon as a mere filtering apparatus (*colatorium*) for the removal of the bile which existed preformed in the blood. As, however, pathological anatomy came to be more and more studied, it could not escape the observations of such men as Morgagni[3], Boerhaave and his learned commentator van Swieten[4], that many cases of jaundice exist which are obviously caused by a mechanical obstruction to the flow of bile into the duodenum, as by calculi and morbid growths obstructing, or pressing upon, it. Without denying, or even doubting, that the specific biliary constituents normally exist preformed in the blood, and that the function of the liver is to excrete them, they recognised two great varieties of jaundice, viz. a jaundice due to obstruction and a jaundice due to non-elimination. This view has found supporters up to the present day, and it is only within a comparatively recent period[1] that facts have accumulated which prove that jaundice is always due to an obstruction to the normal efflux of already secreted bile—an obstruction which may affect the flow of bile along the minutest hepatic ducts.

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[1] H. Tappeiner, 'Ueber die Aufsaugung der gallensauren Alkalien im Dünndarme,' *Sitzungsber. d. Wiener Acad. d. Wissenschaft.* Vol. LXXVII. (1878), Abth. III.

[2] Fr. Glissonii in inclyta Cantabrigiensi Academia Medicinae Professoris Anatomia Hepatis. Hagae-Comitis. Apud Arnoldum Leers. Anno 1681... '*Verum si hepar spectemus......dicendum profecto fuerit hepar in eum finem a natura institutum esse, ut sanguinem a bile defaecatum reddat*' (p. 411).

[3] Morgagni, *De sedibus et causis morborum.*

[4] Ger. L. B. van Swieten, *Commentaria in Hermanni Boerhaavi Aphorismos de cognoscendis et curandis morbis.* Tomus III. Lugd. Batav. MDCCLIII. 'Semper autem supponit (icterus) vel impeditam secretionem bilis a sanguine venae portarum, vel impedimentum tollens liberum exitum bilis secretae in intestinum duodenum...... Verum ingens varietas icteri est ratione causae, quae secretionem bilis vel ejus liberum exitum in duodenum impedit' (p. 127).

The experiments of Kunde and of Moleschott, had shewn that after the extirpation of the liver of frogs, the blood and tissues of these animals are free from all traces of the specific biliary constituents. In spite of these facts, which have received remarkable confirmation and extension, the assumption was made by some (Budd, Harley) that the liver only forms the bile acids, whilst it excretes from the blood ready formed bile colouring matters. Relying on the altogether erroneous assertion that the bile acids are absent from the urine in cases of jaundice in which no obvious obstruction exists, a supposed distinction (based on the non-elimination of bile acids) was attempted to be established between these cases and cases of jaundice in which the existence of an obstruction was obvious.

Frerichs' doctrine of 'polycholia'.

Whilst admitting the already proved fact that the bile acids and bile colouring matters are formed in the liver, Frerichs[2] advanced the theory that, in spite of absence of all obstruction to the outflow of bile, jaundice may arise from the reabsorption of bile already excreted into the intestine. Normally, according to Frerichs, the constituents of the bile are in great part reabsorbed from the intestine, and then undergo in the economy oxidations, by which they are destroyed. If, however, the quantity of bile secreted is greater than can be disposed of in this way, the biliary constituents will accumulate in the blood, stain the tissues, and be excreted in the urine, in short all the phenomena of jaundice will be induced by a condition which may be termed 'polycholia.' The views of Frerichs were supported by many altogether erroneous facts as, for instance, that by the action of sulphuric acid on the bile acids, bile pigments can be produced, and that when the bile acids are injected into the blood *they are con-*

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[1] The development of our knowledge on this subject will be appreciated by the reader who refers to the article Jaundice by the late Dr Murchison in Quain's *Dictionary of Medicine*, London, 1882. The theoretical explanations of cases of 'jaundice independent of mechanical obstruction of the bile-duct' given by this distinguished physician are already as obsolete as are those advanced by Morgagni, Boerhaave and van Swieten.

[2] Fr. Th. Frerichs, *Klinik der Leberkrankheiten*. In zwei Bänden, Braunschweig, 1858. Refer to Vol. I. p. 94 *et seq*, 'Der verminderte Verbrauch, der geringere Umsatz der Galle im Blute.'

verted into bile pigments[1]. Frerich's observation was, however, correct that in certain cases of jaundice in which no obstruction can, *prima facie*, be detected, the bile secretion is at first increased. The *polycholia*, which he described as resulting from certain icterogenic agents, is a precursory, or concomitant, phenomenon, but not the efficient cause, of the jaundice.

'*Does a Hæmatogenic, as distinguished from a Hepatogenic, Jaundice exist?*'

Icterogenic Poisonous Agents.

The observations of Kühne.

We have stated that Frerichs, from the experiments which he had made with Städeler, was led to conclude that the bile acids could be converted into bile colouring matters by the action of chemical agents, and that a similar conversion occurred in the economy; the latter conclusion was drawn from the fact that when decolourized bile was injected into the circulation, the urine, subsequently excreted, contained bilirubin. In a remarkably interesting paper, published in 1858, Kühne[2], whilst confirming the accuracy of the last experiment, pointed out that the salts of the bile acids act in the same way as decolourized bile, and, further, that they possess the power of dissolving the coloured blood corpuscles. He was led to conclude that the bilirubin excreted in the urine was derived not from bile acids, as Frerichs had supposed, but from the hæmoglobin thus set free in the blood, an explanation which appeared plausible enough when we consider that the researches of Virchow and others (see p. 316) had already established that bilirubin (hæmatoidin) can be, and actually is formed from the blood colouring matter when this is locally extravasated. In pursuance of his researches, Kühne injected a solution of the blood colouring matter into the circulation of dogs, but found that the urine excreted contained hæmoglobin or its derivatives, but no bile colouring matter. If, however, a very small quantity of the salt of a bile acid were injected together with the solution of oxy-hæmoglobin, bilirubin was excreted in the urine.

The observations of Kühne seemed to afford a new explanation for one group of cases of jaundice which had been, until then, explained by the old theory of non-elimination or by the more recent hypothesis of Frerichs. The cases to which we refer are particularly those in which jaundice, or at least the elimination of bilirubin, occurs as a consequence of the action of medicinal or poisonous agents, as when chloroform or ether are introduced into the blood, or when an animal is poisoned with phosphorus or arseniuretted hydrogen. In some of these cases, the agent able to lead to the elimination of bilirubin is known to be able to effect a disintegration or solution of the coloured blood corpuscles. With these cases of jaundice due to well-defined toxic agents, it was natural to connect the cases of jaundice occurring occasionally in connection with certain animal poisons, as that of snake bite: with certain zymotic diseases, such as remittent and intermittent fevers: or in connection with acute yellow atrophy of the liver, a disease presenting a remarkable resemblance in its phenomena to slow poisoning by phosphorus and some other agents.

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[1] Frerichs u. Städeler, 'Ueber die Umwandlung der Gallensäuren in Farbestoff,' Muller's *Archiv*, 1856, pp. 56—61.

[2] Kühne, 'Beiträge zur Lehre von Icterus, eine physiologisch-chemische Untersuchung.' Virchow's *Archiv*, Vol. XIV. (1858), pp. 310—356.

The researches of Kühne thus rendered it probable that in addition to an *hepatogenic* jaundice, there might exist a *hæmatogenic* jaundice, perfectly explicable without recourse to the altogether untenable hypothesis that normally the bile colouring matters exist preformed in the blood, and are merely excreted by the liver. Captivating though the new theory at first appeared, the progress of research has shewn it to be incorrect, and has forced us to the conclusion, expressed at the outset of this chapter, that all cases of jaundice are due to reabsorption of bile already formed in, and by, the liver.

In the first instance came a series of researches which, indirectly, led to grave doubts being entertained whether free hæmoglobin existing in the blood is converted into bilirubin. Max Hermann[1] had found that the injection of water into the blood of dogs led to the excretion of bile pigment in the urine, and had explained the phenomena, on Kühne's hypothesis, as due to a conversion of hæmoglobin into bilirubin, independently of any hepatic action. J. Steiner[2], however, on repeating these experiments with rabbits, was only able to discover bile colouring matter twice in twenty-four experiments, the exceptional results being explained by Naunyn[3] by the fact that fasting animals excrete bile colouring matter in the urine.

Experiments of Tarchanoff and Vossius.

Subsequently Tarchanoff[4] injected solutions of crystallised oxy-hæmoglobin into the jugular vein of dogs, and collecting the urine as it flowed through cannulæ tied into the ureters found that, for about two hours or more, after the injection, the urine contained hæmoglobin in solution, but no trace of bile colouring matter, though subsequently the latter made its appearance.

Tarchanoff, further, found that when oxy-hæmoglobin was injected into the veins of dogs with biliary fistulae, the quantity of bilirubin excreted in the bile increased. To elucidate the latter fact, he injected solutions of bilirubin into the veins of dogs, and found that in this case also the amount of bilirubin excreted in the bile increased. From these researches, Tarchanoff was led to support the theory of the possibility of the origin of bilirubin within the blood itself, at the expense of blood colouring matter set free.

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[1] Max Hermann, 'De effectu sanguinis diluti in secretionem urinae.' Dissert. Inaug. Berolini, 1859.

[2] J. Steiner, 'Ueber die hämatogene Bildung des Gallenfarbstoffes.' *Archiv f. Anat. u. Phys.* 1873, p. 160.

[3] Naunyn, 'Beiträge zur Lehre von Icterus.' *Archiv f. Anat. u. Physiol.* 1868, pp. 438—440.

[4] Joh. Fürst Tarchanoff, 'Ueber die Bildung von Gallenpigment aus Blutfarbstoff im Thierkörper.' Pflüger's *Archiv*, Vol. IX. (1874), pp. 53—65; 'Zur Kenntniss der Gallenfarbstoffbildung.' *Ibid.* pp. 329—334.

Vossius[1], repeating the experiments of Tarchanoff, confirmed the statement that when bilirubin is introduced into the circulation, the quantity of colouring matter excreted in the bile increases, though he was unable to observe this increase when hæmoglobin was injected. The thorough investigation of Städelmann[2] on this subject thoroughly confirmed, however, the fact that not only does the bilirubin of the bile increase when bilirubin is introduced into the blood, but also when hæmoglobin is injected into it. It yet remained, however, to discover whether this conversion of hæmoglobin into bilirubin occurs in the blood itself, and whether the process can go on independently of the liver.

Researches of Städelmann, Afanassiew, Minkowski and Naunyn.

In very beautiful investigations on the action of toluylendiamin, $[C_6H_3(CH_3)NH_2)_2]$ and of arseniuretted hydrogen[3], Ernst Städelmann shewed that both these agents often induce hæmoglobinuria and jaundice in the lower animals and that this jaundice, which is at first accompanied by an increased flow of bile (polycholia), and afterwards by a diminished flow (acholia), is almost certainly of hepatogenic origin[4]. Subsequently Afanassiew[5] shewed that, under the influence of the poisons employed by Städelmann, there is developed interstitial hepatitis as well as glomerulo-nephritis, and Minkowski and Naunyn discovered that the polycholia which follows poisoning with arseniuretted hydrogen "goes hand in hand with the appearance of cells in the liver which enclose numerous blood corpuscles and with the transformation of the hæmoglobin which these cells contain. Thus bile colouring matter is formed within the cells which enclose blood corpuscles[6]".

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[1] Vossius, *Quantitative spectralanalytische Bestimmung des Gallenfarbstoffes in der Galle.* Giessen, 1879. See also note 1, p. 367.

[2] Dr Ernst Städelmann, 'Zur Kenntniss der Gallenstoffbildung' (Aus dem Lab. der med. Klinik in Königsberg). *Archiv f. exp. Pathol. u. Pharmak.* Vol. xv. (1882), pp. 337—363.

[3] The fact that jaundice occurs in cases of poisoning by arseniuretted hydrogen in man had been long known. Naunyn, in experiments of which the results were first published in Städelmann's paper, was the first to induce icterus in the lower animals by the inhalation of AsH_3.

[4] Ernst Städelmann, 'Toluylendiamin und seine Wirkung auf den Thierkörper,' *Archiv f. exp. Pathologie und Pharmak.* Vol. xiv. (1881), pp. 231—287 and 422—450; 'Die Arsenwasserstoffvergiftung,' *Ibid.* Vol. xvi. (1883), pp. 221—255.

[5] M. Afanassiew, 'Ueber die pathologisch-anatomischen Veränderungen in Nieren und Leber bei einigen mit Hämoglobinurie und Icterus verbundenen Vergiftungen.' Virchow's *Archiv,* Vol. xcviii. (1884), pp. 460—500.

[6] O. Minkowski und B. Naunyn, 'Ueber den Icterus durch Polycholie und die Vorgänge in der Leber bei demselben.' *Archiv f. exp. Pathol. u. Pharmak.* Vol. xxi. (1886), pp. 1—33. Refer to Sect. 4, 'Ueber die Vorgänge in der Leber bei der (Arsenwasserstoff) Polycholie (p. 19—30).

The conclusive experiments of Minkowski and Naunyn[1].

Stern[2] had shewn that when, in doves, all the blood-vessels going to the liver are tied, as well as the bile-ducts, no bile colouring matter accumulates in the blood and tissues: a result which absolutely proved the incorrectness of Harley's views that the bile colouring matter, unlike the bile acids, is not formed in, but is merely excreted by the liver.

In doves, however, the secretion of urine is completely arrested by the ligature of all the vessels supplying the liver, whereas in ducks and geese, as Minkowski and Naunyn discovered, the secretion continues after the operation. Applying the method first employed by Stern to these animals, with the addition that the liver was subsequently extirpated, they found that if the whole of the liver had been removed, the urine remained free from biliary constituents. Having determined that normal ducks and geese after the inhalation of arseniuretted hydrogen excrete bile colouring matters in the urine, they proceeded to expose ducks and geese from which they had removed the liver to the same agent. In the time which intervened between the poisoning and the death of the bird, urine was excreted which contained hæmoglobin, *but which was free from bile colouring matters*. They thus succeeded in demonstrating that in the most typical cases of supposed hæmotogenic jaundice, the excretion of bilirubin is necessarily connected with, and dependent on, the liver.

In a sense, however, these cases of jaundice induced by icterogenic drugs differ from the ordinary run of cases of jaundice, in that the changes induced in the blood are probably the starting point of the hepatic changes which lead to the subsequent absorption of bile colouring matters and which are the cause of the polycholia which distinguishes them in their earlier stages.

As was previously stated, some (Leyden[3], Budd, Harley) who held the opinion that two great divisions of cases of jaundice existed, believed that they could be discriminated one from the other by determining whether the urine contained bile acids as well as bile colouring matters, the former being said to be only excreted in jaundice from obstruction. As a matter of fact the discovery of bile acids in urine does not admit of being satisfactorily made by the simple employment of Pettenkofer's reaction, and the scientific proof of the presence of bile acids requires operations of considerable complexity. But Städelmann[4] has conclusively proved that, in the jaundice occasioned by icterogenic drugs, the urine often contains bile acids as well as colouring matters, whilst Naunyn[5] discovered them in two cases of pyaemic jaundice in which no obstacle to the flow of bile into the intestine existed, and in which the liver was not more deeply bile stained than the other organs.

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[1] See note 6, p. 364.

[2] Hans Stern, 'Beiträge zur Pathologie der Leber und des Icterus. 1. Ueber die normale Bildungsstätte des Gallenfarbstoffes.' *Archiv f. exp. Pathol. u. Pharmak.* Vol. XIX. (1885), pp. 39—59.

[3] Leyden, *Beiträge zur Pathologie des Icterus.* Berlin, 1866.

[4] Städelmann, *op. cit.* *Archiv f. exp. Path. u. Pharmak.* Vol. XVI. p. 221.

[5] Naunyn, 'Beiträge zur Lehre vom Icterus.' *Archiv f. Anat. u. Phys.* 1868, p. 438 *et seq.*

Does a 'Urobilin Jaundice' exist?

It has long been known[1] that cases of jaundice occasionally occur in which the urine, though of dark colour, does not exhibit Gmelin's reaction. Some of these cases are to be explained by the fact that when stained with bile colouring matter, the skin retains its jaundiced tint for some time; in transitory cases of jaundice it does so for a considerable time after the liquor sanguinis and urine have become free from bilirubin. There are, however, some cases of slight and persistent jaundice in which the urine does not exhibit Gmelin's reaction, but contains abnormal quantities of urobilinoid bodies[2]. These have been called cases of 'Urobilin icterus' (Ictère hémaphéique of Gubler and Dreyfuss-Brissac). Quincke[3], who has investigated this subject carefully, concludes that the jaundiced colour of the skin never depends upon urobilin (a self-evident proposition, seeing that urobilin possesses no such tinctorial power as would enable it to stain the tissues), but that the urobilin found in the urine in these cases is due to a transformation in the urine of normal bile colouring matter. On examining the blood serum he always found it to contain bilirubin. According to Frerichs, jaundiced urine occasionally does not exhibit Gmelin's reaction when first passed, but does so after exposure to air (?). Hence he surmised the existence of certain chromogens, convertible by oxidation into normal bile colouring matters.

SECT. 2. THE MODIFICATIONS IN CHEMICAL COMPOSITION WHICH THE BILE EXHIBITS IN DISEASE.

Our knowledge of the changes which the bile undergoes in disease is remarkably scanty; this necessarily follows from the fact that we have, in the human subject, no means of obtaining the secretion during life, and that such data as we possess are almost entirely derived from analyses of bile obtained post mortem.

The presence in the bile of oxy-haemoglobin and its derivatives: 'haemoglobinocholia'.

In discussing the action of icterogenic poisonous agents we have referred to the fact that, in the first instance, these agents lead to the setting free of the oxy-hæmoglobin which the red blood corpuscles contain. It has been found that under these circumstances hæmoglobin finds its way into the bile. Filehne[4], who gives the name of '*Hæmoglobinocholia*' to this condition, has found that it is set up in the rabbit when this animal is poisoned by the following substances: phenylhydrazin, toluylendiamin, the derivatives of anilin, pyrogallol, potassium chlorate, &c.

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[1] Frerichs, *Klinik d. Leberkrankheiten*, Vol. I. p. 106.

[2] C. Gerhardt, 'Ueber Urobilinurie.' *Wiener med. Wochenschrift*, 1877, no. 24.

[3] H. Quincke, Beiträge zur Lehre vom Icterus. 4 'Ueber den sogenannten Urobilin-icterus.' Virchow's *Archiv*, Vol. xcv. (125—140).

[4] W. Filehne, 'Der Uebergang von Blutfarbstoff in die Galle bei gewissen Vergift-

Vossius[1] had already twice observed the passage of hæmoglobin into the bile of the dog as a result of the injection of water. Wertheimer and Meyer[2] likewise observed the passage of haemoglobin into the bile of dogs when these animals are poisoned by means of anilin and toluidin, as well as when death is induced by cooling the body. It would appear, however, from the subsequent researches of the authors, that the phenomena are not as constant in the dog as in the rabbit[3]. The elimination, by the kidneys, of the hæmoglobin which has passed into solution in the liquor sanguinis appears, in this animal, to be so rapid that its diffusion into the bile does not occur[4]. Probably, hæmoglobinocholia will be found to exist in pernicious anæmia and in early stages of acute yellow atrophy.

The presence of albumin in the bile.

In hyperæmia of the liver resulting from an obstructed venous circulation, albumin, occasionally but by no means always, is found in the bile[5]. In Bright's disease and in some cases of fatty liver (?), and sometimes after injection of water into the blood, the bile is said to contain albumin[6].

The presence of sugar in the bile.

The bile is said often to contain large quantities of sugar in cases of diabetes. According to Claude Bernard the injection of sugar into the blood leads to the secretion of a saccharine bile.

The presence of urea in the bile.

Excess of urea has been found in the bile after death from Bright's disease and cholera.

The presence of leucine and tyrosine in the bile.

Leucine and tyrosine have been discovered in the bile of patients who have died from acute yellow atrophy of the liver.

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ungen und einigen anderen (blutschädigenden) Eingriffen.' Virchow's *Archiv*, Vol. CXVII. (1889), p. 415—417.

[1] Adolf Vossius, 'Bestimmungen des Gallenfarbstoffes in der Galle.' *Archiv f. exp. Pathologie u. Pharmak.* Vol. XI. (1879) 426 *et seq.*

[2] E. Wertheimer and E. Meyer, 'De l'apparition de l'oxyhémoglobine dans la bile et de quelques caractères spectroscopiques normaux de ce liquide.' *Archives de Physiologie*, Juillet, 1889, p. 438 *et seq.*

[3] E. Wertheimer and E. Meyer, 'De quelques faits nouveaux relatifs au passage de la matière colorante du sang dans la bile.' *Archives de Physiologie*, 1 Avril, 1890, p. 425 *et seq.*

[4] See a criticism of Wertheimer and Meyer's first paper by Filehne in a second paper by this author entitled, 'Der Uebergang vom Hämoglobin in die Galle.' Virchow's *Archiv*, Vol. CXXI. (1890), p. 605.

[5] Frerichs, 'Die Stauungshyperämie der Leber,' *Klinik der Leberkrankheiten*, Vol. I. p. 372 and 373.

[6] Gautier, *Chimie Biologique*. Paris, 1892, p. 586.

The absence of bile colouring matters from the bile.

Cases have been recorded where the bile found in the gall bladder after death has been colourless. Some of these have been doubtless cases of the 'hydrops cystidis felleæ' which will be referred to below, the fluid found in the gall-bladder being a secretion of its mucous membrane and not true bile. Ritter[1] has published analyses of colourless, or nearly colourless, bile, in which, the other biliary constituents being present, the bile colouring matters alone were absent, or nearly so. In certain of these cases there was fatty degeneration of the liver, though absence of biliary pigment is by no means characteristic of this condition. The greatest interest attaches, however, to the observations of Noël Paton and Balfour who found, in their case of biliary fistula (p. 275), that the bile always became markedly paler during pyrexia and on several occasions was quite colourless. When the patient was suffering from acute tonsilitis, the bile was totally destitute of colouring matter. 'Throughout the course of the feverish days, in the early morning, when the temperature was lowest, the bile was always well coloured, and it was towards the afternoon, when the temperature rose, that the diminution in the pigment was to be observed[2].'

Summary of the changes observed in the bile, classified under particular diseases.

During pyrexia, the quantity of bile is diminished and the colouring matter, as above stated, may diminish to such an extent that the liquid may be colourless[3].

In congestion of the liver due to venous obstruction, the bile sometimes, though not invariably, contains albumin.

In fatty degeneration of the liver, the bile has been noticed to be deficient in colouring matter, though this is by no means always the case. In amyloid degeneration of the liver, Hoppe-Seyler, on one occasion, found the bile highly pigmented and containing a large quantity of solid matters, of which the larger part was insoluble in alcohol[4]. In acute yellow atrophy, the bile, like the other fluids of the body, contains leucine and tyrosine.

In diabetes, the bile contains sugar. In uraemia, whether due to affection of the kidneys or to cholera, the amount of urea in the bile is increased. In cholera, the mucous membrane of the gall bladder may or may not be affected. In the latter case, the gall bladder contains a 'rice-water' liquid, i.e. a white turbid liquid mixed with flakes of detached epithelium, resembling the liquid which is found in the intestinal canal. In the former case, the gall bladder contains a dark coloured and very concentrated bile, concerning which it is impossible to say whether it is secreted in the condition in which it is found, or whether it has become concentrated in its passage along the biliary ducts[5].

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[1] Ritter, *Comptes Rendus*, Vol. LXXIV. p. 813, and *Journal de l'Anatomie et de la Physiologie*, 1872, p. 181. The reader may also refer to a paper by V. Hanot entitled, 'Notice pour servir à l'histoire de l'acholie.' *Comptes rendus de la Société de biologie*, 1884, pp. 41 and 336.

[2] D. Noël Paton and J. M. Balfour, *op. cit.* (Refer to p. 211).

[3] Noël Paton and J. M. Balfour, *op. cit.*, (p. 211 and 212 refer also to p. 204).

[4] Hoppe-Seyler, *Physiolog. Chemie*, p. 318.

[5] Hoppe-Seyler, *Physiolog. Chemie*, p. 316.

THE SECRETION OF THE GALL-BLADDER IN SO-CALLED HYDROPS CYSTIDIS FELLEÆ.

In the records of cholecystotomy a large number of cases occur in which the gall-bladder having been opened, in order if possible to remove a calculus obstructing the neck of the bladder or the biliary passages, it was found full of a colourless, generally highly viscous, liquid, of alkaline reaction. The calculus is, in these cases, very frequently impacted in the cystic duct and acts as a valve, permitting the expulsion of bile from the gall-bladder but preventing its entrance. The colourless liquid found in these cases is not a decolourised bile, but the secretion of the mucous membrane of gall-bladder. When complete occlusion of the gall-bladder exists, it may become enormously distended with this fluid, and there is then established the condition known as dropsy of the gall-bladder, or *hydrops cystidis felleæ*. It would be a great mistake to suppose that the mucous secretion which fills the gall-bladder in these cases represents, either qualitatively or quantitatively, its normal secretion. Frerichs, Naunyn, and others have found indeed that in such cases the epithelium of the gall-bladder has changed in character. Referring to them, Naunyn says, "das Epithelium verliert den Character des Cylinderepithels. Ich fand in mehreren Fällen, statt seiner, einen aus viel grösseren kubischen Zellen bestehenden, Epithelbelag. Das Gleiche fanden Pittres u. A. vor mir[1]."

Glisson[2] and de Graaf[3] were the first to examine the fluid under discussion, though the first chemical analysis of it was published by Frerichs[4], who, in one case, found it to contain, in 1000 parts, 982·7 parts of water and 17·3 parts of solid matters. The organic matters (mucin, &c.) amounted to 16·0, the alkalies to 0·6 and the earths to 0·7 parts for 1000. In some cases of this kind, if the gall-bladder be opened and a fistula established, the secretion of liquid continues. Two such cases have been observed by Mr Mayo Robson of Leeds. The fluid was, in the first instance, examined by Birch and Spong[5]. One of these cases was afterwards much more satisfactorily investigated by Mr Mayo Robson himself[6].

The case was that of a woman, aged 32 (weighing 48·6 kilos.), who was operated on in January, 1884, for distended gall-bladder due to gall-stones. A fistula having been established, a constant flow of somewhat viscid liquid was set up; this was assumed to be the unmixed secretion of the gall-bladder, as complete occlusion of the cystic duct existed and as no trace of bile could be detected in the secretion. The average quantity of fluid secreted in 24 hours was found to be 71·3 c.c. The following are the results of the observations made on April 29, 1884.

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[1] B. Naunyn, *Klinik der Cholelithiasis* p. 106. Compare also Frerichs' remarks (*Klinik d. Leberkrankheiten*, Bd. II. p. 449).

[2] Glisson, *Anatom. Hepatis*, Cap. 39.

[3] de Graaf, *Tractatus de succo pancreatico*, Cap. 8.

[4] Frerichs, *Klinik d. Leberkrankheiten*, Bd. II. p. 449.

[5] Birch and Spong, *Journal of Physiology*, Vol. VIII. p. 378.

[6] Mayo Robson, *Proceedings of the Royal Society*, Vol. XLVII. (1890), pp. 499 *et seq.*

Volume of secretion in 24 hours	72 c.c.
Sp. gr.	1009·5
Reaction.	Alkaline.
Solid matters in 1000 parts	15·36
Water	984·64

Organic matters, chiefly mucin, in 1000 parts	6·72
Chlorides equal to NaCl	5·73
Sodium carbonate	2·20
Other salts, containing phosphates, potassium salts, &c.	0·71

In accordance with the opinion already expressed, it appears, however, to the Author, that we cannot from the study of the above and similar cases form any accurate idea of the secretion of the normal gall-bladder. The above analysis represents the characters of the liquid found in typical cases of *hydrops cystidis felleæ* and was probably the product of a mucous membrane of which the secreting epithelium no longer possessed its normal characters.

Empyema of the gall-bladder.

As a result of an infective cholecystitis, empyema of the gall-bladder has been observed, *i.e.* the gall-bladder becomes distended with a purulent fluid. Naunyn, who has investigated such cases, has found the purulent fluid to contain *Bacterium coli commune*[1].

SECT. 3. THE INFLUENCE OF DRUGS ON THE SECRETION OF BILE. CHOLAGOGUES.

In discussing the action of so-called icterogenic poisonous agents, we incidentally pointed out that they appear to lead, in the first instance, to an increase of the quantity of bile secreted, to a true polycholia. We have now to refer briefly to researches which have been made with the view of determining whether medicinal agents exist which exert a direct action on the secretion of bile, especially whether drugs exist which may correctly be termed hepatic stimulants or cholagogues. The fact that certain drugs when administered to man induce copious bilious stools has, from time immemorial, been supposed to indicate that they possess a truly cholagogue action, but the conclusion is one which cannot logically be drawn from the premises.

"The clinical observer," says Professor Rutherford, to whose researches we owe the greater part of our knowledge on this subject, "has supplied most valuable information regarding the power of various substances to increase the amount of bile in the dejections. He observes dejections of a clay colour, he gives five grains of calomel, and further observes that in some cases the dejections thereafter assume their natural appearance. He cannot be certain of the manner in which this result is brought about. For anything he knows, it might be occasioned (1) by stimulation of the hepatic secreting apparatus; or (2) by the stimulation of the muscular fibres of the gall-bladder and larger bile-ducts—to wit—the bile-expelling apparatus; or (3) by removing a catarrhal or congested state of the orifice of the common bile-duct, or of the general extent of the larger bile-ducts; or (4) by removing from the intestine substances which had been passing therefrom into the portal vein and depressing the action of the hepatic cells; or (5) by stimulating the intestinal glands, and thus producing drainage of the portal system, whereby the 'loaded' liver might possibly be relieved[1]."

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[1] B. Naunyn, 'Die infectiöse Cholecystitis und das Empyem der Gallenblase,' *Klinik d. Cholelithiasis*, p. 105.

In truth, the investigation of cholagogues is one of the most difficult in the whole range of pharmacology, for the various methods of research which have hitherto been employed are all, more or less, open to objections which compel the utmost caution in drawing inferences from them, and have led to the most contradictory results.

The first observations on the cholagogue action of a drug had reference to calomel and were performed on dogs with biliary fistulæ. "By this method, Nasse[2], Kölliker and Müller[3], Scott[4], severally made observations on a single dog with reference to the effect of calomel on the biliary secretion. Being in some measure contradictory, the subject was in 1866 taken up by a committee, of which the late Professor Hughes Burnett was chairman and reporter[5]. Professor Arthur Gamgee and the Author were the two junior members of the committee upon whom devolved the task of performing the experiments. The investigation was laborious and lasted two years[6]." The conclusions arrived at by this committee were that calomel, mercuric chloride and taraxacum do not increase the flow of bile but probably act on the bile expelling apparatus.

"In 1873 Röhrig[7] observed the rate of biliary flow from temporary fistulæ in fasting curarised dogs, before and after the injection of purgative agents into the stomach or intestine. He found that large doses of croton oil greatly increased the secretion of bile and that a similar effect, though to a less extent, was produced by colocynth, jalap, aloes, rhubarb, senna, and sulphate of magnesia—the potency of these agents as stimulants of the liver being in the order mentioned. He found, moreover, that castor oil had little effect and that calomel, while it seldom recalled the biliary secretion after it had ceased, nevertheless somewhat augmented it when taking place slowly. Röhrig's statement with regard to calomel does not differ much from that made by Hughes Burnett's committee, but nevertheless he did find that certain purgative agents, when given to fasting animals with temporary biliary fistulæ increased the biliary secretion, while the committee found that in *non-fasting* animals with permanent biliary fistulæ purgative action, induced by podophyllin, calomel, &c., diminished the amount of bile secreted in the twenty-four hours[1]."

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[1] W. Rutherford, 'An Experimental Research on the Physiological Action of Drugs on the Secretion of Bile.' (From the *Transactions of the Royal Society of Edinburgh*, Vol. XXIX.). Edinburgh, 1879.

[2] J. H. Nasse, 'Commentatio de bilis quotidie a cane secretæ copia et indole.' Quoted by Rutherford.

[3] Kölliker und Müller, 'Beitrag zur Lehre von der Galle.' *Würzburg. Verhandlungen*, Vol. V. (1855). Quoted by Rutherford, p. 231.

[4] G. Scott, 'On the influence of mercurial preparations on the secretion of bile.' Beale's *Archives of Medicine*, Vol. I. p. 209.

[5] *British Association Reports*, 1868.

[6] Rutherford, *op. cit.* p. 136.

[7] Röhrig, 'Experimentelle Untersuchungen über die Physiologie der Gallenabsonderung.' *Wiener med. Jahrbücher*, 1873, p. 240.

Rutherford's researches. Subsequently[1] Professor Rutherford resumed the investigation of this subject and in 1879 published the elaborate memoir already referred to. In his researches, like Röhrig, he determined the rate of flow of the bile from temporary fistulæ in fasting curarised dogs. It is impossible to give here the whole of the (fifty-four) conclusions at which Professor Rutherford arrived, but the following brief summary comprises his most interesting results: drugs appear undoubtedly to exist which may be called hepatic stimulants, in so far that they increase the flow of bile in the unit of time, and of these some exert a powerful and some only a feeble stimulant action. Of these drugs, some are not only cholagogue but exert a more or less powerful stimulant action on the intestinal glands, while others have no action on the latter. The following well-known drugs are placed by Rutherford amongst the powerful hepatic stimulants:

Sodium phosphate (i. s.).
Mercuric chloride.
Ipecacuanha.
Colchicum (i. s.).
Jalap (i. s.).
Aloes.
Colocynth (i. s.).
Sodium benzoate.
Sodium salicylate.

The drugs in the above list to which the letters (i. s.) are appended, are powerful intestinal as well as hepatic stimulants. Ipecacuanha as well as sodium benzoate and salicylate are examples of hepatic stimulants almost without action on the intestinal glands.

The following well-known drugs are hepatic stimulants, though their action is much feebler than those first referred to:

Rhubarb.
Acid. nitro-hydrochl. dil.

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[1] Rutherford, *op. cit.* p. 136.

Calomel, according to Rutherford, stimulates the intestinal glands but not the liver, whilst mercuric chloride is a powerful hepatic stimulant exerting but little action on the intestine. The latter statement must be held to apply to medicinal doses, as in poisonous doses corrosive sublimate, as is well known to the toxicologist, is one of the most powerful of intestinal irritant poisons.

"Purgation produced by purely intestinal stimulants, such as magnesium sulphate, gamboge and castor oil, diminishes the secretion of bile." "When a substance—e.g. podophyllin—which powerfully stimulates the intestine as well as the liver, is given in too large a dose, the bile-secretion may never be increased, and though it should be increased in the first instance, it is soon diminished as the excitement of the intestinal mucous membrane extends downwards and implicates a larger and larger number of glands[1]." Amongst the results which Rutherford obtained is to be mentioned that he found that bile, in sufficient doses, exerted a cholagogue action.

Researches subsequent to those of Rutherford.

Since the publication of Prof. Rutherford's researches, the action of cholagogues has been investigated anew with the aid of animals and human beings with permanent biliary fistulæ. For the most part, these results have led to the denial that any true cholagogues exist. Amongst those, however, who have obtained positive cholagogue results is Rosenberg[2], who experimenting on two dogs with permanent biliary fistulæ found that olive oil, bile, and sodium salicylate exert a truly cholagogue action.

From the observations of Battistini[3] on two dogs with permanent biliary fistulæ, it results that santonin is a cholagogue of very decided activity, and his results have been confirmed by Marfori[4].

With the exception of the observations just referred to nearly all others performed since the publication of Rutherford's researches have led to negative results. Thus Baldi[5] in the case of two dogs with biliary fistulæ found that, *with the exception of bile itself*, no agent introduced into the stomach or intestine affected the secretion of bile. Mayo Robson[6] performed a series of experiments in his case of biliary fistula (p. 275) in the human subject which seemed to shew that none of the reputed cholagogues exerted any action whatever, and Paschkis[1] and Nissen[2] experimenting on dogs with biliary fistulæ obtained negative results with all cholagogues, *the bile alone excepted.* Besides Nissen, Mandelstamm[3], Müller[4], Loewenton[5] and Glass[6] have, in Dorpat under E. Stadelmann's direction, determined the action of various medicinal agents on the biliary secretion of dogs with complete fistulæ and consuming a constant quantity of water and solid food. Their results are opposed to those of Rutherford and tend to negative the existence of any drugs exerting an action as hepatic stimulants. All these negative results notwithstanding, we[7] are not of the opinion that the experiments of Röhrig and of Rutherford are *necessarily* incorrect, because investigations performed by a different method have led to contradictory results. It has already been pointed out that all facts combine to prove the existence of the so-called circulation of the bile and it therefore follows that a man or an animal from whose alimentary canal all bile is diverted, by means of a biliary fistula, is so far removed from the normal condition that pharmacological experiments of the nature of those we are discussing cannot be held to be quite conclusive.

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[1] Rutherford, *op. cit.* p. 256.

[2] J. Rosenberg, 'Ueber die cholagoge Wirkung des Olivenöls in Vergleich zu der Wirkung einiger anderen cholagogen Mittel.' Pflüger's *Archiv*, Vol. XLVI. (1889), pp. 334—366.

[3] Battistini, 'Einfluss des Santonins auf die Gallenausscheidung.' *Untersuch. zur Naturlehre von Moleschott*, Vol. XIII. pp. 414—431. Abstracted in Maly's *Jahresbericht*, Vol. XV. (1886), p. 316.

[4] Marfori, 'Sulla pretesa azione colagoga della Santonina.' *Annali di Chimica e di farmacologia.* Ser. 4, Vol. X. p. 153. Abstracted in Maly's *Jahresbericht*, Vol. XIX. (1890), p. 289.

[5] Dario Baldi, 'Recherches expérimentales sur la marche de la sécrétion biliaire.' *Archives italiennes de Biologie*, 1883, p. 389.

[6] Mayo Robson, *op. cit. Proceedings of the Royal Society*, Vol. XLVII. (1890), p. 499.

In the present condition of the question, it appears to the Author to be desirable that a renewed investigation of the subject be carried out, with the aid of dogs with Schiff's amphibolic biliary fistulæ. The results obtained in this way would be free from most of the objections which can be advanced against observations carried out either by the method of Röhrig and of Rutherford, or on dogs or human beings with permanent fistulæ of the ordinary kind.

Sect. 4. The Elimination of Medicinal and Poisonous agents in the Bile.

It was Orfila, the founder of modern toxicology, who directed attention to the fact that the majority of metallic poisons are taken up by the liver and either retained by that organ or excreted in the bile, so that in the investigation of cases of poisoning by arsenic, antimony, mercury, copper, lead and zinc, the examination of the liver is of particular importance. Copper, in particular, has been found to be an almost constant, though doubtless an adventitious, constituent of liver and the bile, and its presence is to be accounted for by the fact that the food of man, especially the vegetable food, always contains traces of copper[1]. Ellenberger and Hofmeister found 0·02 and 0·04 per cent. of CuO in the bile of the sheep[2]. Zinc has also been frequently found in the liver and tissues of human beings and animals[3].

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[1] H. Paschkis, 'Ueber Cholagoga.' *Med. Jahrbücher*, 1884, p. 159. Quoted by Neumeister.

[2] W. Nissen, 'Experimentelle Untersuchungen über den Einfluss von Alkalien auf Sekretion und Zusammensetzung der Galle.' Dorpat, Diss. Inaug. 1889. Maly's *Jahresbericht*, 1890, p. 280.

[3] Mandelstamm, 'Ueber den Einfluss einiger Arzneimittel auf Secretion und Zusammensetzung der Galle.' *Inaug. Diss.* Dorpat, 1890.

[4] Müller, 'Ueber den Einfluss einiger pharmakologischer Mittel auf Secretion und Zusammensetzung der Galle.' *Inaug. Diss.* Dorpat, 1890.

[5] A. Loewenton, 'Experimentelle Untersuchungen über den Einfluss einiger Abführmittel und der Clysmata auf Secretion und Zusammensetzung der Galle, sowie deren Wirkung bei Gallenabwesenheit im Darme.' *Inaug. Diss.* Dorpat, 1891.

[6] J. Glass, 'Ueber den Einfluss einiger Natronsalze auf Secretion und Alkaliengehalt der Galle.' *Inaug. Diss.* Dorpat, 1892, and *Archiv f. exp. Path. u. Pharmak.* Vol. xxx. (1892), pp. 241—274.

[7] 'Aeltere Beobachter, wie Röhrig und Rutherford, scheinen sich in dieser Beziehung getäuscht zu haben. Aus einer Reihe neuerer Untersuchungen hat sich ergeben, dass *sogenannte Cholagoga* nicht existiren, wenn man nicht gewisse Gallenbestandtheile selbst als solche bezeichnen will.' Neumeister, *Lehrbuch der phys. Chemie.* Jena, 1893, p. 155.

Claude Bernard found that sulphate of copper, iodide of potassium, spirit of turpentine and grape sugar, when injected into the blood, rapidly pass into the bile. Amongst other substances which are excreted in the bile are: potassium chlorate (Prévost and Binet[4]); salicylic acid. Diakonow[5] shewed that when indigo-carmin is injected into the jugular vein of the rabbit, after the method of Chrontschewsky[6], as well as when it is injected subcutaneously or introduced into the stomach, it is rapidly excreted in the bile. Peiper[7] made the interesting observation that in dogs with permanent biliary fistulæ, when iodide of potassium was introduced in large doses (5 grms) into the rectum, it could only be detected in the bile 5 or 6 hours after the injection. Sodium salicylate was found within half an hour. Sulphocyanide of potassium also passed into the bile, but neither potassium ferrocyanide nor ferricyanide.

Wertheimer[8] has shewn that the sodium compound of phyllocyanic acid, which is an immediate derivative of chlorophyll-green, when introduced into the blood, is rapidly excreted by the bile. Amongst substances which are not excreted in the bile may be mentioned the following: alcohol (Mosler): atropia, muscarin, strychia (Prévost and Binet): kairin and antipyrin (Prévost and Binet).

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[1] The reader is referred to the exceedingly complete and interesting Monograph by Dr A. Tschirch, Professor of Pharmacognosy, Pharmacy and Toxicology in the University of Bern, entitled *Das Kupfer vom Standpunkte der gerichtlichen Chemie, Toxicologie und Hygiene*. Stuttgart, Verlag von Ferdinand Enke, 1893, pp. 138. In this work will be found *inter alia* a complete account of the literature relating to the distribution of copper throughout the animal and vegetable kingdom.

[2] Ellenberger und Hofmeister, *Archiv f. wissensch. u. prakt. Thierheilkunde*, 1883, p. 325. Quoted by Tschirch, *op. cit.* p. 19.

[3] G. Lechartier and F. Bellamy, 'Sur la présence du zinc dans le corps des animaux et dans les végétaux.' *Comptes Rendus*, Vol. LXXXIV. p. 687. F. Raoult and H. Breton, 'Sur la présence ordinaire du cuivre et du zinc dans le corps de l'homme.' *Comptes Rendus*, Vol. LXXXV. p. 40.

[4] Prévost and Binet, 'Recherches expérimentales relatives à l'action des médicaments sur la sécrétion biliaire et à leur élimination par cette sécrétion.' *Revue médicale de la Suisse romande*. No. 520, Mai, 1888.

[5] Diakonow, 'Ueber das Verhalten der Indigoschwefelsäure im thierischen Organismus.' Hoppe-Seyler's *Med.-chem. Untersuchungen*, Berlin, 1866, pp. 245—254.

[6] Chrontschewsky, Virchow's *Archiv*, 1866.

[7] E. Peiper, 'Uebergang von Arzneimitteln aus dem Blute in die Galle nach Resorption von der Mastdarmschleimhaut aus.' *Zeitschrift f. klin. Med.* Vol. IV. (1882), p. 402 *et seq.*

[8] M. E. Wertheimer, 'Sur l'élimination par le foie de la matière colorante verte des végétaux.' *Archives de Physiologie*, Jan. 1893, pp. 124—130.

Passage of pathogenic micro-organisms into the bile.

The normal bile is sterile (Gilbert et Girode)[1]. "At a time when every drop of the circulating blood is teeming with micro-organisms there may not be the slightest transit of them into the urinary and biliary fluids then secreted" (Sherrington[2]). On the other hand, as the researches of a number of observers have conclusively proved, when pathogenic organisms exist in the blood they tend, after a time, to pass into the bile and urine, 'and their escape into the secreta is sometimes accompanied by the escape of actual blood,' sometimes by the appearance of albumin, although blood be absent. Sherrington is of the opinion that in the normal condition of the hepatic and renal membranes, a passage of micro-organisms through them cannot occur, and that it is in all probability only after the soluble poisons produced by the infection have had time to act upon them that the membranes become pervious to germs.

The following micro-organisms have been found to make their way into the bile:—the bacillus of glanders, *B. mallei* (Ferraresi and Guarnieri): the bacillus of typhoid fever, *B. typhi abdominalis* (Trambusti and Maffucci): the spirillum of cholera, *B. choleræ asiaticæ*, (Nicati): *B. coli commune* (Blachstein): the bacillus of anthrax, *B. anthracis* (Oemler, Straus and Chamberland, Sherrington): *Staphylococcus pyogenes aureus* (Pernice and Scagliosi): *B. pyocyaneus* (Pernice and Scagliosi, Sherrington): Friedlander's *pneumococcus* (Pernice and Alessi, Sherrington): *B. murisepticus* (Sherrington)[3].

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[1] Gilbert et Girode. *Comptes Rendus de la Société de Biologie*, 1890, No. 39, and 1891, No. 11.

[2] C. S. Sherrington. 'Experiments on the escape of bacteria with the secretions.' Reprinted from *The Journal of Pathology and Bacteriology*. Edinburgh and London, Young J. Pentland, Feb. 1893.

[3] The references to all the authorities here referred to will be found either in Sherrington's paper or at page 47 of Naunyn's *Klinik der Cholelithiasis*. Leipzig, 1892.

CHAPTER VII.

THE FORM, STRUCTURE AND CHEMICAL COMPOSITION OF BILIARY CALCULI. CHOLELITHIASIS AND THE THEORIES ADVANCED TO EXPLAIN IT.

SECT. 1. THE FREQUENCY OF OCCURRENCE, THE FORM, THE CLASSIFICATION AND STRUCTURE OF GALL-STONES[1].

IN a preceding Chapter of this Book we have already referred incidentally (see p. 316) to biliary calculi and have pointed out that one, though much the less frequent, variety contains considerable quantities of the compound of bilirubin and calcium, nearly always mixed, however, with more or less cholesterin, and containing small quantities of little investigated derivatives of bilirubin. A second variety is composed almost entirely of cholesterin.

Though biliary calculi may be found in the intra-hepatic biliary ducts, much the larger number occur in the gall-bladder, and where calculi are found in the cystic or common bile-duct they have almost invariably migrated from their seat of formation in the gall-bladder.

Frequency of gall-stones. Number found.

The frequency of the occurrence of biliary concretions led the great French pathologist Cruveilhier to remark, 'La production des calcules biliaires est une des lésions les plus communes de l'espèce humaine[2]'. Charcot illustrates the accuracy of Cruveilhier's statement by telling us that, in his experience, in about one-fourth of the autopsies of the aged women dying in the great hospital of La Salpétrière, concretions have been found in the gall-bladder[1].

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[1] *Early history of Gall-stones.* Gall-stones were first noticed in the year 1565 by J. Kentmann, of Dresden, who communicated his observations to Conrad Gessner, who published them in his work entitled *De omnium rerum fossilium genere*, Tigur. 1565. Amongst the earlier accurate observers of gall-stones were Vesalius, Fallopius, Glisson, Sydenham, Boerhave and van Swieten, Sauvages. The first chemical examination of gall-stones was made by Galeatti (*Comment. Acad. Scient.* Bonon. 1748, t. I. p. 354). Fourcroy and Thenard, after discovering cholesterin, made the first really scientific examination of biliary concretions. The fact that the colouring matters of bile in biliary calculi are combined with calcium was discovered by Bramson. For references, and other information, on the subject of the history of gall-stones the reader is referred to Frerichs, *Klinik d. Leberkrankheiten*, Vol. II. pp. 466 and 467.

[2] Cruveilhier, *Traité d'anatomie pathologique*, t. II. p. 167.

The number of calculi which are found in the gall-bladder varies greatly. Occasionally, though rarely, a single calculus is found, though more commonly the number varies between 5 and 30. As many as 3000 calculi were found in one gall-bladder by Morgagni, and 7802 by Otto (Breslau Museum).

Physical characters of gall-stones.

However few or numerous the calculi contained in a gall-bladder, usually they all possess the same chemical composition, the same structure, the same volume, the same colour. Exceptions to this rule are rare.

The size of biliary calculi varies greatly, the average being about the size of a hazel-nut. Meckel described a biliary calculus which was 15 centimetres long and 6 broad, and which only weighed 30·3 grms.

When biliary calculi occur singly they are rounded or ovoid. When they attain a very large size they are pyriform, as they mould themselves to the shape of the cavity in which they are formed. Multiple calculi usually exhibit facets. These facets are produced by the mutual pressure of the concretions one against the other whilst these are of yet soft consistence, and not by a process of attrition.

The colour of biliary calculi presents great varieties. Those which are composed of nearly pure cholesterin are throughout white and sometimes transparent. Others, which are also composed of cholesterin, possess a more or less coloured and opaque exterior, which is sometimes yellow and sometimes greenish. The colour depends especially on the presence of particular stages of oxidation of the biliary colouring matters.

Biliary calculi have a very low specific gravity, which is, however, always higher than that of water or bile. Soëmmering and some other authors have fallen into error in asserting that biliary calculi occasionally float in water and bile. When recent, biliary calculi always sink in these liquids. Sometimes, however, biliary calculi are found in museums which, having become dry, float. If these are, however, plunged in water or bile for some time, bubbles of air are seen to rise and the calculus, acquiring its original density, sinks to the bottom of the liquid.

Structure of gall-stones.

"As a rule, biliary calculi present (1) a central nucleus: (2) a middle zone, which is in general composed of multiple concentric lamellæ, formed by radiating crystalline pyramidal masses of cholesterin: (3) a laminated external layer or shell.

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[1] Charcot, 'Leçons sur les Maladies du Foie et des Reins faites à la Faculté de Médecine de Paris.' Recueillies et publiées par Bourneville et Sevestre, Rédacteurs du *Progrès Medical*, Paris, 1877. The Author has, in his descriptions of calculi, made great use of the large stores of information contained in the lectures devoted to the subject of biliary lithiasis.

"The nucleus commonly presents a brownish-black or greenish colour, and is usually formed of a combination of biliary pigments with calcium. The nucleus is sometimes solid, sometimes hollowed as a result of a process of desiccation, in which case a more or less subdivided cavity exists. Sometimes, the nucleus contains concrete mucus (Ch. Robin, Frerichs), or shrivelled epithelial cells (Frerichs). Finally, the nucleus has sometimes, though very rarely, been found to contain foreign bodies, the existence of which Cruveilheir incorrectly denied. In the famous often-quoted case of Lobstein, which he figured in the Atlas accompanying his *Traité d'Anatomie Pathologique*, a desiccated *Ascaris lumbricoides* formed the nucleus of the calculus. In the subject which furnished this calculus, thirty other ascarides were found in the biliary passages.

"As examples of foreign bodies constituting the nuclei of biliary calculi may be cited the following:—(1) the case of Naucke, in which a needle two centimetres long formed the centre of a biliary concretion of the size of a walnut; (2) the case recorded by Buisson, in which the centre of the nucleus consisted of a small aggregation of blood; (3) a case recorded by the same observer, in which the nucleus of a biliary calculus in an ox was formed by a *Distoma hepaticum;* (4) I would remind you of the fact that Thudichum has found the nuclei of a certain number of biliary calculi obtained from the same gall-bladder to be formed of branching filaments, evidently representing moulds of the interior of small intra-hepatic biliary ducts and which appear to have played the part of centres of formation of the concretions[1].

"Little remains to be said concerning the structure of the middle layer of gall-stones. This layer is usually, as has been previously stated, composed of crystals of cholesterin and presents a radiated aspect. Sometimes the radiations are interrupted by concentric striæ or layers which cut the crystalline pyramids perpendicularly to their long axis. The middle layer is either quite white or transparent or, on the contrary, more or less coloured. In the latter case, the biliary pigment has intermingled in varying proportions with the cholesterin, which in the former case was free from it. Rarely, the middle layer, though composed of cholesterin, presents a soapy uniform aspect, without stratification and without any evidence of crystalline structure.

"The laminated external shell is observed in the larger number of cases. Yet, as has been stated, it is sometimes absent. In such cases, the bases of the crystalline pyramids project to the very external surface as mammillated protuberances. When an external layer exists, it is almost always very clearly distinguished from the middle zone, by its colour, its stratified appearance, and its consistence. It is sometimes composed of cholesterin arranged in thin layers, which, when seen in section, appear to be separated by striæ of biliary pigment. In other cases, the external layer is due to the compound of biliary pigment and calcium forming one or several layers of greater or less thickness and possessed of a brown or green colour. Finally, the external layer may present strata of calcium carbonate separated by pigmentary deposits[1]."

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[1] The accuracy of this observation is denied by Naunyn, 'Thudichum hat behauptet, dass sich in dem Centrum der meisten Blasengallensteine Abgüsse von Lebergallengängen fänden. Er meinte, dass diese Gallengangscylinder gewöhnlich den Krystallisationskern für die Gallenblasenconcremente bildeten. Nach den Abbildungen, die Thudichum von seinen Gallengangscylindern giebt, kann ich nur sagen, dass ich sie nie gesehen habe.' *Klinik d. Cholelithiasis*, p. 49.

Naunyn's classification of biliary calculi.

Naunyn, in his monograph on cholelithiasis, adopts the following classification of biliary calculi: (1) pure cholesterin calculi: (2) stratified cholesterin calculi: (3) the common biliary calculi, that is the usually yellow or whitish-brown calculi which are found in considerable numbers in the gall-bladder, which commonly are facetted and are often of a soft or friable consistence, when first obtained: (4) the mixed bilirubin-calcium calculi, which occur singly or to the number of two or three in the gall-bladder or the large biliary passages and which when multiple may present facets. They are either composed entirely of a reddish-brown or dark-brown mass, or they possess a central, laminated, cholesterin nucleus. Even those parts of the stone which appear to consist almost entirely of bilirubin contain as much as 25 per cent. of cholesterin: (5) pure bilirubin-calcium stones. These are never large, varying from the size of grains of sand to that of peas. For the most part, they have the consistence of wax, though a variety occurs which is harder; the latter, which are usually very minute and never larger than peas, are of a steel-grey or blackish colour and possess a metallic lustre.

The small calculi are composed in great part of bilirubin-calcium, though they always contain biliverdin-calcium, besides bilifuscin and bilihumin; they very rarely contain bilicyanin. The calculi belonging to this class contain very small quantities of cholesterin; sometimes barely recognisable traces.

(6) Rarer forms of biliary calculi, which include some already referred to in Charcot's description, viz. (*a*) amorphous and imperfectly crystallised cholesterin stones of small size: (*b*) calculi containing calcium carbonate. This salt is often present in large quantities in addition to bilirubin-calcium. Naunyn has often found the nucleus of the common gall-stones to be composed of agglomerations of spheres and warty masses of calcium carbonate: (*c*) concretions with heterogeneous bodies as a nucleus, or concretions which may be termed conglomerate stones: (*d*) casts of the hepatic ducts[2].

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[1] Charcot, *op. cit.* p. 122 *et seq.*

[2] B. Naunyn, *Klinik der Cholelithiasis* (Mit 3 farbigen und 2 lichtdruck Tafeln). Leipzig, Verlag von F. C. W. Vogel, 1892. Refer to p. 1—6. The Author wishes to

"The pure bilirubin-calcium stones are found not only in the gall-bladder, but also in the intra-hepatic ducts. The habitual tenants of the gall-bladder are the common mixed cholesterin-calculi" (Naunyn). It is to be noted, however, that small concretions of pure cholesterin may, and do, originate in the intra-hepatic bile-ducts.

SECT. 2. ENUMERATION OF THE CONSTITUENTS AS YET FOUND IN GALL-STONES. THE PIGMENTS WHICH ARE ONLY FOUND IN GALL-STONES (BILIFUSCIN AND BILIHUMIN).

We have already referred to the fact that cholesterin and the calcium compound of bilirubin constitute the most important constituents of biliary concretions, though the latter is often mixed with considerable quantities of calcium carbonate.

In addition to bilirubin in combination with calcium, calculi which contain this compound may contain biliverdin, bilicyanin, choletelin and imperfectly known bodies, described as bilifuscin and bilihumin, which are also for the most part combined with lime. The bilirubin-calcium calculi nearly always contain copper (which Naunyn thinks probably exists as a bilirubin-copper compound), besides iron. Frerichs[1] examined and described calculi of bilirubin-calcium which contained globules of metallic mercury, and his observations on this point have been confirmed by several observers (Beigel[2], Lacarterie[3], Naunyn[4]).

It is to be noted that neither free bilirubin nor the salts of the bile-acids occur in gall-stones; the traces of these substances which are discoverable are derived from the bile with which the gall-stones are permeated.

In rare cases, biliary calculi have made their way into the urinary passages and uric acid has then been found as a constituent[5].

Similarly, when gall-stones have sojourned for some time in the intestines they may be coated with phosphate of calcium and magnesium, as well as with calcium carbonate[6].

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acknowledge his great indebtedness to this able, interesting, and admirably illustrated work, the appearance of which has marked a new era in our knowledge of cholelithiasis.

[1] Frerichs, *Klinik d. Leberkrankheiten*, Vol. II. pp. 474 and 475.

[2] Beigel, *Wiener med. Wochenschr.* 1856, No. 15.

[3] Lacarterie, *Gazette méd. de Paris*, 1827, quoted by Charcot, *op. cit.* p. 131.

[4] Naunyn, 'So beschrieb Frerichs Gallenconcremente (die ich übrigens selbst untersuchen konnte) welche aus Gallenfarbstoffkalk bestanden und Kügelchen metallischen Quecksilbers enthielten,' *loc. cit.* p. 7.

[5] Güterbock, *Berlin. klin. Wochenschr.* 1871, Nos. 49 and 51, and Virchow's *Archiv*, Vol. LXVI. (1876), p. 273. The reader is referred to an account of the literature of cases of this kind in the learned work by Professor Courvoisier of Basel, entitled *Casuistisch-statistische Beiträge zur Pathologie und Chirurgie der Gallenwege*, Leipzig, 1890. The cases yet recorded will be found at page 362, under the heading, 'Ulcerative Perforationen der Gallenwege in die Harnwege.'

[6] Charcot, *op. cit.* p. 133.

Bilifuscin $C_{32}H_{40}N_4O_8$?

By this name Städeler[1] designated a constituent of gall-stones which, contrasted with bilirubin, is very sparingly soluble in chloroform, but is soluble in absolute alcohol. When bilirubin-calcium gall-stones have been treated with hydrochloric acid and then extracted with chloroform (see page 316), the first chloroform extract contains some bilifuscin. When evaporated to dryness, the residue yields the latter substance to absolute alcohol. The greater part of the bilifuscin is to be found in the residue from which chloroform has extracted bilirubin. If this be dried and treated with absolute alcohol, the latter dissolves bilifuscin. The solution is evaporated to dryness, extracted with boiling water, and the insoluble residue again dissolved in as small a quantity as possible of absolute alcohol. From its solution in the latter, the colouring matter is precipitated by the addition of large quantities of ether. It is again dissolved in alcohol and the solution evaporated to dryness.

Bilifuscin is described as a dark-brown body easily soluble in alcohol, glacial acetic acid, and solutions of the alkaline hydrates. It is sparingly soluble in chloroform and is insoluble in water and ether. Its solutions are brown, with a shade of olive-green. Its ammoniacal solution is precipitated by calcium chloride, insoluble brown flakes of bilifuscin-calcium being obtained. Bilifuscin does not exhibit Gmelin's reaction.

Bilihumin ?

By this term is designated the insoluble colouring matter which is left after decomposing bilirubin-calcium calculi with dilute hydrochloric acid and extracting with chloroform, absolute alcohol and ether. The body, which is doubtless a mixture of derivatives of bilirubin, does not exhibit Gmelin's reaction.

Naunyn[2] seems to consider bilihumin to be almost a characteristic of the small biliary calculi which take their origin in the intrahepatic ducts, and which are to be distinguished from concretions of inspissated bile by their containing the higher oxidation products of the bile-colouring matter (to wit, biliverdin, bilicyanin and choletelin) as well as bilihumin-like bodies. It is very hard to understand how oxidations can take place within the hepatic ducts.

Biliprasin ??

The colouring matter described under this name by Städeler is believed to be a mixture of bilifuscin and biliverdin.

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[1] Städeler, 'Ueber die Farbstoffe der Galle,' *Annalen d. Chemie u. Pharm.* Vol. CXXXII. (1864), pp. 323 *et seq.*

[2] Naunyn, 'Die Entstehung der Bilirubin-Kalksteinchen in den Gallengängen der Leber.' *Op. cit.* p. 27.

Bilicyanin, Choletelin.

According to the observations of Heynsius and Campbell, which have been confirmed by subsequent observers (Naunyn), these two bodies, which, as we have seen (pp. 328—330) are products of the oxidation of bilirubin and biliverdin, occur in certain of the biliary concretions of man.

SECT. 3. THE MODE OF FORMATION OF GALL-STONES.

It is a common, indeed a general belief, among those who have devoted the greatest thought and study to the subject of cholelithiasis, that all circumstances which tend to hinder the flow of bile favour the formation of gall-stones, and, indeed, that a retarded movement of bile is an essential condition to the formation of these concretions. It is obvious that, *cæteris paribus*, the liver will be rapidly and effectively drained of its bile in proportion as the respiratory movements, especially the diaphragmatic and abdominal respiratory movements, are active, and conversely that all conditions which tend to limit the respiratory movements will tend to a stasis of bile in the intra-hepatic biliary ducts. The remarkable frequency of biliary concretions in women, as compared with men, has been explained (Naunyn) by the fact that their respiratory movements (costal type of respiration) are less favourable to the compression of the liver and the efflux of its bile than the characteristically diaphragmatic type of respiration in man; that pregnancy must, of necessity, by impeding to a remarkable extent the diaphragmatic and abdominal respiratory movements, greatly increase the tendency to biliary stasis, a tendency perhaps aided by other conditions which specially affect women, as *e.g.* tight lacing and sedentary occupations.

In an investigation carried out, at Naunyn's suggestion, Schröder[1] in the pathological institute of Strassburg, found gall-stones in 4·4 p$\frac{o}{o}$ of all male subjects and 20·6 of all female subjects whose bodies were examined. Among 115 female subjects with gall-stones, 99 had *with certainty* borne children!

The frequency with which gall-stones are found in the gall-bladder increases remarkably with age, as is shewn in the annexed table, which exhibits Schröder's results.

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[1] Schröder, quoted by Naunyn, *Klinik der Cholelithiasis*, p. 87. The only reference given by Naunyn is the following, 'Schröder, Strassburger Doctor-Dissert. Wird, 1892, oder 93 publicirt.'

Age	Number of Autopsies	Number of bodies in which gall-stones found	Percentage of autopsies in which gall-stones were found
0—20	82	2	2·4
21—30	188	6	3·2
31—40	209	24	11·5
41—50	252	28	11·1
51—60	161	16	9·9
60 and over	258	65	25·2

This increase in the frequency of the occurrence of gall-stones in old people has been supposed by some to be due to their bile containing an excess of cholesterin, but this explanation, as will be subsequently argued, is an untenable one. As age advances and the muscular activity of the body diminishes, as the habits become more and more sedentary, the respiratory activity will certainly be diminished. If, then, there be any truth in the view that the liver is rapidly and effectively drained of its bile in proportion as the respiratory movements are active, and that a slow movement of the bile is a primary condition for the formation of gall-stones, it will follow that in old age one, at least, of the conditions favourable to the formation of gall-stones exists.

But the investigations of Charcot and Pitres[1] have shewn that the unstriped muscular fibres which exist in the walls of the biliary passages undergo remarkable atrophy in old age, so that the probability of the expulsion of any calculi which may be formed is much smaller than at earlier periods of life. Thus, perhaps, we may in part account both for the remarkable increase in the number of gall-stones found after death as age advances, as well as for the comparatively small number of cases in which gall-stones give rise in old people to attacks of biliary colic.

But in what manner can an arrested or retarded movement of the bile lead to the formation of biliary calculi? If, in truth, such a connection, as has been generally surmised, actually exists, it is, in all probability, an indirect one, the retarded flow favouring the action of agents which play a more direct part in the process.

We have seen that biliary calculi in man are in the large majority of cases composed mainly of cholesterin mixed in some cases with calcium compounds of the bile colouring matters, whilst the concretions formed mainly of the latter compounds are comparatively rare. In order to explain the formation of gall-stones, we shall, therefore, have to inquire into the circumstances which lead to the origin and separation of cholesterin and the calcium compound of bilirubin.

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[1] Charcot et Pitres, see Charcot, *Leçons sur les Maladies du Foie et des Reins*, p. 143.

As has previously been stated (p. 339), cholesterin is chiefly held in solution in the bile by the alkaline salts of the bile acids, though the neutral fats and the alkaline salts of the fatty acids also possess the power of dissolving considerable quantities.

From the experiments of Happel[1], recorded by Naunyn, sodium glykocholate or taurocholate, when existing in solutions containing from 0·2 to 2·5 per cent. of these bodies, are able, at temperatures of 37°—38° C., to dissolve about one-tenth of their weight of cholesterin. Soap in dilute solutions is able to dissolve about half its own weight and olein dissolves 5 p.c. of its weight of cholesterin.

Bilirubin and calcium both exist in the bile. The former appears to be held in solution by the alkaline salts of the bile acids which, in addition, possess the power of hindering its precipitation by calcium salts, unless these are added in great excess (Naunyn[2]). The addition of albumin to a solution of sodium glykocholate, holding bilirubin and calcium salts in solution, causes, however, a separation of the insoluble calcium and bilirubin compound (Naunyn[3]).

Frerichs' theory of the formation of gall-stones[4].

Frerichs assumed that a stagnation of bile in the gall-bladder was the first condition for the formation of gall-stones. Under the influence of mucus secreted by the gall-bladder, he believed that the salts of the bile acids then underwent decomposition[5] and the reaction of the bile became acid: consequently, the cholesterin, and the bilirubin which had been held in solution by the bile salts, separated, the former in a crystalline condition, the latter partly in a crystalline condition, but chiefly as the insoluble calcium compound (Bramson). He drew attention to the important part played in this process by lime salts, which, he shewed, are secreted by the mucous membrane of the gall-bladder; this he repeatedly had found incrusted with numberless crystals of calcium carbonate. Frerichs believed that, in order to lead to the formation of biliary concretions, the precipitated bodies must needs remain some time in the gall-bladder and require the co-operation of the elements resulting from catarrh of its lining membrane (mucus, epithelium ?), though he gives no details as to the process.

In criticising Frerichs' theory from the present stand-point of science it is at once obvious that such an acid fermentation as he assumed, leading to a decomposition of the salts of the bile acids could not originate spontaneously, but presupposes the intervention of some active agent, presumably of a pathogenic organism.

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[1] Happel's experiments appear to have been performed at Naunyn's instigation and are recorded in his *Klinik d. Cholelithiasis*, p. 16.

[2] Naunyn, *op. cit.* p. 18.

[3] *Ibid.* p. 19.

[4] Frerichs, *Klinik der Leberkrankheiten*, Braunschweig, 1861. 'Die Entstehung der Gallenconcremente,' Vol. II. pp. 484—487.

[5] 'Stockung und Zersetzung der Galle ist also die erste Ursache der Concrementbildung,' Frerichs, *op. cit.* p. 485.

As the bile has been shewn to be normally sterile, the mere slowing of its current would be insufficient to induce the changes which Frerichs assumed. But even were such an acid decomposition, as he imagined, to occur, there is no evidence that it would lead to a separation of the cholesterin of the bile, inasmuch as such a separation does not occur when bile decomposes, through exposure to atmospheric germs, and acquires an acid reaction.

Naunyn's theory of the production of gall-stones.

When discussing (p. 340) the probable origin of the cholesterin of the bile, we drew attention to the views of Naunyn, who has advanced the remarkable hypothesis that it is not a product removed by the liver from the blood, but that it takes its origin in the epithelial cells of the gall-bladder and biliary passages. Whilst we advanced arguments which appear to us to prove that such a view is inadmissible in the case of the normal cholesterin of the bile, we would point out that these arguments do not invalidate the possibility, nay the certainty, of the local production of cholesterin, as a result of morbid processes having their seat in the epithelial cells lining the mucous membrane of the biliary passages.

We have drawn attention to the observations of Frerichs which merely confirmed and extended those made before him by Cruveilhier[1], as to the local excretion of calcium salts by the mucous membrane of the gall-bladder, when this becomes the seat of inflammatory action. Naunyn and his pupils have supplemented our knowledge of this subject by shewing that the amount of calcium in the bile is not affected by the amount taken into the body and existing in the blood[2,3]. The only constituent of gall-stones, indeed, which, according to Naunyn, is influenced by the food is bilirubin, the amount of which seems to be larger when the diet is abundant than when it is scanty.

The most careful inquiries have revealed that neither hereditary nor acquired diathesis, neither nationality nor dietetic habits, appear to affect the incidence of cholelithiasis. Rich and poor, fat people and spare, the gouty *bon vivant* and the abstemious peasant, all suffer alike from gall-stones. Such being the case, must we not seek for a local pathological process affecting in the first instance the mucous membrane of the biliary passages, and leading secondarily to the formation of gall-stones?

The essence of Naunyn's theory of cholelithiasis consists in assuming that it is due to an infection of the gall-bladder and biliary passages, by the migration into them of organisms existing in the duodenum, of which some exert a pathogenic action on the mucous membrane, this migration being facilitated when the rate of flow of the bile is diminished.

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[1] Cruveilhier, *Traité d'Anatomie pathologique*, Vol. II. p. 190.

[2] Naunyn, *Klinik d. Cholelithiasis*, 'Die Kalkausscheidung in der Galle,' p. 14.

[3] Dr Ludwig Jankau, 'Ueber Cholesterin- und Kalkausscheidung mit der Galle' (Aus d. med. Klinik d. Univ. Strassburg). *Archiv f. exp. Path. u. Pharmak.*, Oct. 1891, pp. 237—246.

As was formerly said, the normal bile is sterile, a fact first demonstrated, in the case of the rabbit, by Netter in 1884[1], and confirmed in the case of man by Gilbert and Girode[2] in 1890.

Netter and Martha, Brieger, Leyden and others had found in purulent processes affecting the biliary passages of man, in addition to other organisms (*Staphylococci, Streptococci*), a bacillus which subsequent investigation has proved to be the *Bacterium coli commune*. The same bacillus was afterwards found and cultivated by Levy in a hepatic abscess, consecutive to gall-stones, occurring in Naunyn's clinique. In five cases of cholelithiasis, in which an acute cholecystitis had supervened, Naunyn, by puncturing the gall-bladder during the life of the patient, discovered the same bacillus.

The organism thus discovered is eminently pathogenic and experiment has shewn that when introduced into the biliary passages of dogs, after ligature of the common bile-duct, it induces acute infection and rapidly kills the animal, whilst if a similar culture of the bacillus is injected without ligaturing the duct, no bad consequences follow and, when the animal is killed after an interval, no abnormal appearance is observed, either in the biliary passages or the hepatic substance.

Naunyn's view is that the *Bacterium coli commune* migrating from the intestine, under the necessary conditions of a retarded or arrested bile-flow, induces an affection of the mucous membrane of the gall-bladder, a 'calculus-forming' catarrh ('steinbildende Katarrh').

When death suddenly occurs in individuals suffering from gall-stones (but not suffering from cholecystitis), the epithelium cells lining the gall-bladder are found to contain fat drops and, besides, so-called myeline masses, with double outlines, which in some cases fill the whole cell. From some of these myelin-laden cells, the masses are seen to protrude and then to become detached, floating away singly, or becoming aggregated into clumps of glassy, structureless, highly refractive, matter. Such glassy, structureless masses as these are actually found floating in the bile in cases such as those we are considering. On the addition of acetic acid, they may be observed, under the microscope, to congeal into a mass of cholesterin crystals. These clumps of cholesterin are, as Naunyn shews, the first rudiments of gall-stones, and accompanying them are exactly similar but harder masses, veritable little calculi. At first, these little calculi have a glassy structure, but sooner or later the cholesterin commences to crystallise[1].

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[1] Netter, quoted by Naunyn, *op. cit.* p. 43.

[2] Gilbert et Girode, *Comptes Rendus de la Société de Biologie*, 1890, No. 39; 1891, No. 11.

Whenever this remarkable development of small calculi from clumps of cholesterin could be observed, Naunyn always found other very minute cholesterin calculi, which, however, contained a central cavity filled with a brown, softish, mass, composed mainly of bilirubin-calcium, and he was able, in the case of these also, to study the process of formation from its very commencement. At first, aggregations of swollen epithelial cells are usually seen, which break down to form a granular, brownish, mass and, in the immediate proximity, similar brown granular masses are seen, around which confluent myelin forms have set, forming a glassy capsule of cholesterin.

It is impossible to reproduce Naunyn's interesting description of the minor variations which may be observed in the mode of origin of calculi and, for a knowledge of these, the reader is referred to his work. It only remains for us to point out how, according to Naunyn's investigations, the calculus grows and is modified. The growth of calculi composed mainly of bilirubin-calcium occurs, doubtless, in consequence of actual precipitation of the compound from the bile, occasioned partly by the pouring out of a secretion rich in lime salts from the walls of the gall-bladder (an event which, as we have seen, always accompanies a catarrhal condition of the gall-bladder), but partly, perhaps, in consequence of the simultaneous passage into the bile of albumin, the presence of which greatly aids the precipitation.

The growth of a calculus through the addition of cholesterin generally occurs, according to Naunyn, by the superposition of, or infiltration by, the masses of cholesterin around it, though where a calculus is surrounded by bile it may also increase in size through the crystallisation of cholesterin which was in solution in that liquid. The infiltration, previously referred to, takes place through minute canals which penetrate from the outer zone into the interior of calculi—so called infiltration canals ('Infiltrationscanäle')—which permit both the primitive soft cholesterin masses and the bile to permeate the concretion.

It must be added that, according to Naunyn, the crystalline structure of cholesterin-calculi is not, in general, due to a primary deposition of cholesterin in the crystalline form, but is due to a secondary process of crystallisation which invades the mass of cholesterin forming the calculus, after this has been deposited.

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[1] The reader is referred to the most beautiful chromolithographs and photo-type engravings illustrating the structure of gall-stones in Naunyn's book.

SECT. 4. RESULTS OF QUANTITATIVE ANALYSES OF THE CHIEF VARIETIES OF BILIARY CALCULI.

I. *Cholesterin Calculi.*

The most complete investigations on the composition of biliary calculi have been made by Ritter[1], who for the purposes of his research made a collection of 6000 specimens.

The maximum and minimum quantities of cholesterin, organic matters other than cholesterin, and mineral matters in his analyses of cholesterin calculi are as follows:

	Maximum.	Minimum.
Cholesterin in 100 parts	98·1	64·2
Other organic matters in 100 parts	27·4	1·5
Mineral matters in 100 parts	8·4	0·4

The largest amount of bilirubin found by Ritter in a cholesterin calculus was 1·2 per cent.

The following are the results of the analysis of a cholesterin calculus made by v. Planta and Kekulé[2]:

Water in 100 parts	4·89
Cholesterin	90·82
Saponifiable fat	2·02
Biliary colouring matter	0·20
Mucin (?)	1·35
Matters soluble in water	0·79
Mineral matters	0·28

II. *Bilirubin-calcium Calculi.*

In a bilirubin-calcium calculus (human), Ritter found traces of cholesterin, 75·2 per cent. of organic matters and 24·8 per cent. of mineral matters.

The following analyses exhibit the composition of a bilirubin-calcium calculus of the ox, made by Maly[3].

Bilirubin in 100 parts	28·10
Fatty matters	5·28
Matters soluble in water	18·09
Phosphates and earths combined with bilirubin	1·41
Insoluble matters and loss	47·13

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[1] Ritter, *Journal de l'Anatomie et de la Physiologie*, 1872, pp. 60 and 181. *Comptes Rendus*, Vol. LXXIV. (1872), p. 813.

[2] v. Planta and Kekulé, *Annalen d. Chem. u. Pharm.* Vol. LXXXVII. p. 367.

[3] R. Maly, 'Zusammensetzung der Ochsengallensteine,' *Jahresb. d. Thier-Chemie*, Vol. IV. (1875), pp. 310—312.

Maly found another calculus of the ox to contain 45 per cent. of bilirubin. These calculi contained no cholesterin.

Phipson analysed a similar concretion from the pig, with the following results:

Water in 100 parts	8·00
Bilirubin	61·36
Ether extract (fat and cholesterin)	1·35
Hyoglycholate of sodium	2·75
Mucin	11·50
Mineral matters	13·65

III. *Calculi rich in Mineral Matter.*

The following is the analysis by Ritter of a calculus weighing 1·36 grms. found in the gall-bladder of an aged woman:

Cholesterin in 100 parts	0·4
Bilirubin	0·6
Biliprasin (?)	0·8
Bilihumin	12·8
Matters soluble in water	2·3
Calcium carbonate	64·6
Calcium phosphate	12·3
Ammoniaco-magnesium phosphate	3·4
Mucin and loss	2·8

CHAPTER VIII.

METHODS FOR THE ANALYSIS OF THE BILE AND BILIARY CALCULI.

WE have in the preceding Chapters treated so fully of the properties and methods of separating the normal constituents of the bile, that it only remains to describe the methods which are employed in the systematic analysis of bile and biliary calculi, and which are of special importance to the physician and the pathologist.

SECT. 1. EXAMINATION OF THE BILE FOR ALBUMIN, OXYHÆMOGLOBIN AND ITS DERIVATIVES, SUGAR, UREA, LEUCINE AND TYROSINE.

Albumin. The bile is cautiously neutralised by means of dilute acetic acid and then boiled, when the production of a coagulum will indicate the presence of albumin.

Sugar. The bile is decolourised by means of animal charcoal, filtered, and the filtrate is tested for sugar, (1) by Fehling's test, (2) by the fermentation test.

Oxy-hæmoglobin and its derivatives. In the case of the presence of sufficient blood-colouring matter, a red colour and the characteristic spectrum of oxyhæmoglobin may be observed. The bile, however, readily decomposes this body, throwing down a precipitate which contains both hæmatin and albuminous substances (Hoppe-Seyler). This precipitate should be dissolved in dilute solution of sodium hydrate when the spectrum of hæmatin in alkaline solution is observed; on treating the solution with ammonium sulphide the spectrum of hæmochromogen (reduced hæmatin) is obtained (see Vol. I. 1st ed. p. 110).

In examining the bile of the ox and of the sheep, when two bands alone are visible, their position should be carefully determined and compared with that of the oxyhæmoglobin bands, before the conclusion is arrived at that this substance is present; the necessity for caution arises from the fact that the two central bands of the so-called cholohæmatin may be distinctly observed before the other bands have become visible.

Urea. The bile is mixed with dry animal charcoal evaporated to dryness, and the residue is dissolved in absolute alcohol. The solution is thoroughly precipitated with anhydrous ether and, after subsidence of the precipitate of salts of bile acids, the clear ethero-alcoholic solution is evaporated to dryness. The residue is dissolved in water. The urea present may then be separated by Drechsel's method of alcoholic dialysis, which was employed by Haycraft in the research on urea in the blood which he made under Drechsel's direction[1]. The urea thus obtained must then be identified.

Leucine and tyrosine. The bile is fully precipitated by the addition first of solution of lead acetate and then of ammonia, and the filtrate from the abundant precipitate which falls is treated with sulphuretted hydrogen. The filtrate from the precipitate of lead sulphide is evaporated on the water bath and set aside to crystallise. The leucine and tyrosine which may separate are identified, separated and treated as described under leucine (pp. 234 and 236).

SECT. 2. QUANTITATIVE DETERMINATION OF THE SPECIFIC GRAVITY, TOTAL SOLIDS, SALTS, MUCOID NUCLEO-ALBUMIN, BILE ACIDS, FATS, SOAPS, CHOLESTERIN, LECITHIN AND BILE-COLOURING MATTERS.

1. Specific gravity. Determine *by means of the specific gravity bottle*, noting carefully the temperature (Vol. I. 1st ed. p. 174 *et seq.*).

2. Total solids and salts. Weigh out exactly about from 5 to 10 grammes of bile and determine the total solids and salts, exactly as described in the case of blood (Vol. I. 1st ed. p. 177—180).

3. The mucoid nucleo-albumin. Treat from 10—30 grammes of bile with five times their volume of absolute alcohol and centrifugalise. When the precipitate has separated in a coherent mass (*i.e.* in about 10 minutes with a velocity of 2000—3000 p. min.), collect it on a weighed filter, the amount of the ash in which is also known. Thoroughly wash the precipitate with absolute alcohol and collect the alcoholic filtrate and washings. The precipitate is then further washed with dilute acetic acid; the filter and precipitate are dried, first at 100° C., then at 110°, and afterwards weighed. Thus are found the amount of mucoid nucleo-albumin together with some insoluble salts and a trace of bile-colouring matters. The filter and precipitate are then ignited and the ash weighed. On deducting the weight of the ash from that of the dried precipitate, the weight of the mucoid nucleo-albumin is obtained.

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[1] In Vol. I. (1st edition), p. 192 (*Haycraft's method*).

4. Determination of neutral fats, soaps and cholesterin.

A quantity of about 10 grammes of bile is treated with alcohol exactly as stated under 3, the precipitate of the mucoid-nucleo albumin being carefully washed with ether. The alcoholic and ethereal solutions are evaporated to dryness and the residue thoroughly extracted with alcohol and ether, filtered, evaporated to dryness and weighed. Thus is found the combined weight of the cholesterin, lecithin and neutral fats. In order to obtain the separate amounts of these constituents, proceed exactly as prescribed for the determination of these bodies in the blood (Hoppe-Seyler's method, Vol. I. 1st edition, p. 187).

5. Determination of the combined salts of the bile acids, of the quantity of the respective acids and of the alkaline metals combined with them.

The determinations under this head necessitate a thorough training in the methods employed and should, with the exception of the first and simplest, not be attempted by inexperienced chemists.

(*a*) The simplest, but only approximate, method of determining the amount of the salts of the bile acids in the bile, is to evaporate a weighed quantity of bile to dryness, after mixing it with pure animal charcoal. The thoroughly dried residue is extracted with boiling ether and afterwards repeatedly with boiling alcohol. The alcohol solutions are filtered, evaporated in a weighed porcelain crucible until the weight of dry residue is constant; thereafter the residue is ignited, the weight of the ash being deducted from that of the total alcoholic residue. Thus we obtain approximately the weight of bile acids.

(*b*) (Hoppe-Seyler's method.) 30 grammes of bile are treated exactly as described under 3 (p. 392); indeed the same quantity of bile which serves to furnish the amount of the mucoid nucleo-albumin will, if not too scanty, suffice for the determinations now being considered.

The mixed alcoholic solution is completely precipitated by adding many times its volumes of anhydrous ether. The precipitate which separates consists principally of the alkaline salts of the bile-acids, but contains also small quantities of the alkaline salts of the fatty acids and of oleic acid, besides sodium and potassium chloride. The precipitate is allowed thoroughly to subside and, after decantation of the alcohol and ether, is dissolved in distilled water; the solution, having been diluted to a known volume or weight, is divided into three parts, of known if not of equal volume or weight; these we shall designate α, β, γ. The fraction α is evaporated to dryness, first on the water bath, then at 110° C., and after weighing is ignited, and the ash is then weighed. In the ash the quantity of chlorine, potassium and sodium are determined by the ordinary methods.

The fraction β serves for the determination of the amount of sulphur, whence the amount of taurocholic acid is determined. It is evaporated to dryness in a silver basin and then ignited with caustic soda and potassium nitrate, or it is treated by Carius' method (heating in a sealed tube with strong pure nitric acid). Whichever method is employed for oxidising the sulphur, the amount of sulphuric acid is determined in the usual manner by precipitating with barium chloride, &c.

The fraction γ serves for the estimation of the glykocholic acid, taurocholic acid and the fatty acids. If necessary, it should be decolourized by means of recently ignited pure animal charcoal, the latter being afterwards thoroughly washed with alcohol and the combined alcoholic fluids concentrated on the water bath and brought up to a known volume. The specific rotation of the alcoholic solution is now determined (see Vol. I. 1st edition, p. 7 *et seq.*).

Either the whole of the alcoholic solution, or a known fraction of it, is now evaporated so as to expel the alcohol; the watery solution of the residue is then placed, *lege artis,* in a thick and hard tube in which have been previously placed at least 5 grms. of dry caustic baryta. The tube is then sealed about a decimeter above the level of the fluid, and, after being allowed to cool, the tube is thoroughly shaken and then heated in the oil bath at 110°—120° C. for ten or twelve hours. The tube is, thereafter, cautiously opened, the liquid is poured into a beaker, the tube thoroughly washed, and CO_2 is then passed through the solution until no further precipitation of barium carbonate occurs. It is then heated to boiling and filtered, at this temperature, through a hot-water funnel.

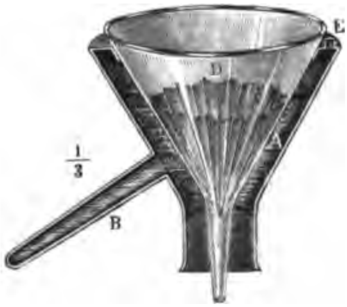

FIG. 23. A HOT-WATER FUNNEL.

The projecting tube B permits of the water being heated to boiling during the progress of filtration, the steam being allowed to escape through E.

The filtrate contains barium cholalate, besides glycocine and taurine, whilst the precipitate consists of barium salts of the fatty acids and of oleic acid, mixed with much barium carbonate. From this precipitate, the fatty acids and oleic acid are obtained by decomposing with dilute hydrochloric acid, repeatedly extracting with ether and evaporating the ethereal solution to dryness. The aqueous solution of barium cholalate, &c. is concentrated to a small volume, the solution not being filtered from the precipitate which separates; ether is added to it and then dilute hydrochloric acid, which throws down cholalic acid. The liquid is allowed, however, to remain exposed to the air for some days so as to permit of the evaporation of the ether. The cholalic acid which has then separated is collected on a weighed filter, washed, dried at 120° C. and weighed. If desired, the filtrate may be freed from barium by means of ammonium carbonate and the amount of ammonia and the sulphur in it (representing the taurine) determined.

Knowing the amount of sulphur, we may calculate firstly the amount of taurocholic acid which it represents, and secondly the amount of cholalic acid which would result from its decomposition, seeing that 100 parts of taurocholic acid, when decomposed, yield 72·22 parts of cholalic acid. If we deduct this quantity of cholalic acid from the total amount which is obtained as a product of the decomposition of the mixed bile acids, we naturally find the amount of cholalic acid which must have existed as glykocholic acid, seeing that 100 parts of cholalic acid represent 113·98 parts of glykocholic acid.

The determination of the rotatory power of the alcoholic solution previously recommended serves to control the results obtained by the method just described.

If a be the observed rotation expressed in degrees for the line D of a column of the solution 1 decimeter thick, and m the amount of taurocholic acid calculated from the weight of the barium sulphate (the Sp. Rot. $(a)D$ of sodium taurocholate in alcoholic solution being $= +25^\circ{\cdot}3$ and of sodium glykocholate $+27^\circ{\cdot}6$), then the amount x of glykocholic acid in the fluid will be found by the following equation:

$$x = \frac{100 . a - m . 25{\cdot}3}{27{\cdot}6}.$$

Determinations of the Colouring Matters of the Bile.

These are only possible by the method of spectro-photometry. As we know the optical constants, of which a knowledge is needed, both of bilirubin and biliverdin, it is perfectly possible to determine the absolute and relative amounts of bilirubin and biliverdin in a bile containing one or other or both of these constituents. The methods of spectro-photometry will be described in all detail in the 2nd edition of Vol. I.

SECT. 3. THE METHODS OF ANALYSING GALL-STONES.

The gall-stone is powdered and the powder is repeatedly treated with boiling water, which extracts the bile with which most calculi are imbibed. The residue is dried and then extracted repeatedly with a mixture of equal parts of alcohol and ether, which removes the cholesterin. The insoluble residue is then treated with dilute hydrochloric acid, when, if calcium carbonate be present, effervescence is observed. The insoluble matter, after treatment with hydrochloric acid, is thoroughly washed with water. After drying, it is treated with chloroform. The method of separation and purification of bilirubin is that described at page 316. The hydrochloric acid solution in the above process will contain any copper which may be present in the concretion and which will be readily discovered by the ordinary tests.

The methods of quantitative analysis to be employed depend somewhat upon the constitution of the calculus. From the hints given above and from the full information given previously as to the determination of the various constituents of the bile which occur in gall-stones, it will be easy to conduct the quantitative analysis of any gall-stone.

CHAPTER IX.

THE INTESTINAL CANAL AND ITS SECRETION. THE INTESTINAL JUICE OR SUCCUS ENTERICUS.

SECT. 1. INTRODUCTORY OBSERVATIONS ON THE STRUCTURE OF THE INTESTINAL TUBE.

The Small Intestine.

THE small intestine is a convoluted tube which commences at the pylorus and ends in the large intestine or colon, the opening between the two being guarded by the so-called *ilio-cæcal* or *ilio-colic valve.* "Its convolutions occupy the middle and lower part of the abdomen and are surrounded by large intestine. They are connected with the back of the abdominal cavity and are held in their position by a fold of the peritoneum, named the mesentery, and by numerous blood-vessels and nerves."

"The small intestine is arbitrarily divided into three portions, which have received different names; the first ten or twelve inches immediately succeeding to the stomach and comprehending the widest and most fixed part of the tube, being called the *duodenum,* the upper two-fifths of the remainder being named the *jejunum,* and the lower three-fifths the *ileum.* There are no distinct lines of demarcation between these three parts, but there are certain peculiarities of connections and differences of internal structure to be observed in comparing the upper and lower ends of the entire tube[1]."

The intestine, small and large, throughout its length from the pylorus to close upon the rectum, possesses the general arrangements of structures which characterise the whole alimentary canal. "A thin outer longitudinal muscular layer, covered by peritoneum, is succeeded by a thicker inner circular muscular layer, and this double muscular coat is separated, by a submucous layer of loose connective tissue, carrying the large blood-vessels, from the mucous membrane; the latter consists of an epithelium lying upon a connective tissue basis of a peculiar nature. A well-developed *muscularis mucosæ,* composed of longitudinal and circular fibres, marks off the mucous membrane proper from the underlying submucous tissue."

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[1] Quain's *Anatomy*, 9th ed. Vol. II. p. 599.

"In the small intestine the outer longitudinal muscular layer is evenly distributed over the whole circumference of the tube and is everywhere much thinner than the inner circular layer, which is the more important of the two. The individual fibre-cells of these muscular layers of the intestine are large and well-developed. In the thin sheet of connective tissue which separates indistinctly the two layers, lies the *plexus of Auerbach*, a plexus of nerve-fibres, for the most part non-medullated, at the nodes of which are gathered groups of very small nerve-cells, the substance of each cell being especially scanty. This plexus supplies the two muscular layers with nerve-fibres. The submucous coat contains, besides blood-vessels and lymphatics, a somewhat similar plexus of nerve-fibres, called the *plexus of Meissner;* from this plexus fine nerve-fibres proceed to the blood-vessels, to the muscularis mucosæ, and possibly to other structures[1]."

The mucous membrane of the small intestine.

"This is thrown into folds which are not, as in the case of the stomach, temporary longitudinal folds, *rugæ*, but permanent transverse folds, the *valvulæ conniventes*, reaching half-way or two-thirds of the way round the tube. Each fold is a fold of the whole mucous membrane, carrying with it a part of the submucous tissue, the latter thus forming a middle sheet between the mucous membrane on the upper surface and that on the lower surface of the fold. The folds, which vary in size, large and small frequently alternating, begin to appear at a little distance from the pylorus; they are especially well-developed just below the opening of the bile and pancreatic ducts, and are continued down to about the middle of the ileum, where, becoming smaller and irregular, they gradually disappear. They serve to increase the inner surface of the intestine and present an obstacle to the too rapid transit of material along the tube."

"Over and above the coarser inequalities of surface caused by these folds, the level of the mucous membrane is broken, on the one hand by tongue-like projections, the *villi* and, on the other hand, by tubular depressions, the *glands* or *crypts of Lieberkühn*[2]."

Description of the glands of Lieberkühn.

"The latter are very much smaller and are more numerous than the former, several crypts being placed in the interval between two villi. Both are found on the projecting valvulæ as well as in the valleys between, and both extend along the whole length of the intestine from the pylorus to the ilio-cæcal valve; but while the villi vary a good deal, being short and few immediately next to the pylorus, very numerous and large in the duodenum and upper part of the intestine, less numerous, smaller, and more irregular in the lower part, the crypts have nearly the same characters and are distributed throughout. Very much as in the case of the stomach, the muscularis mucosæ runs in an even line (except for the sweep of the valvulæ conniventes) at a little distance from the bases of the closely packed crypts, and at a greater distance (viz. the length of the crypts) from the bases of the villi; as we shall see, however, the muscularis mucosæ sends up muscular fibres into each villus."

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[1] Dr M. Foster, *A Text-Book of Physiology*, Part II. p. 441, Macmillan and Co., London and New York, 1889.

[2] *Ibid.* p. 442.

The crypts of Lieberkühn are lined by columnar or rather cubical epithelium which is continuous with that which covers the intestinal surface and the villi.

"They are straight or nearly straight tubes about 400μ long and 70μ broad. The outline is furnished by a very distinct basement membrane, in which nuclei are imbedded at intervals, and this basement membrane is lined with a single layer of short cubical cells, leaving a small but distinct lumen; the cells should perhaps be rather described as somewhat conical, with a broader base at the basement membrane and a narrower apex abutting on the lumen. The cell-body, surrounding a somewhat spherical nucleus, is faintly granular except for a hyaline free border, which however is not so conspicuous or so constant as in the columnar cells of the villi. Similar cells cover the ridges intervening between adjacent glands, and where a villus comes next to a gland the short cubical cells of the gland may be traced into the columnar cells of the villus, the hyaline border becoming more marked and the mucous becoming oval. Among the cubical cells of the gland are to be found, in varying numbers, goblet cells quite similar to those of the villi...."

"Outside the basement membrane between adjacent glands and between the blind ends of the glands and the underlying muscularis mucosæ, is reticular connective tissue, finer and more truly reticular than that of the villi; it is perhaps less crowded with leucocytes. In this reticular tissue run, encircling the glands, capillary blood-vessels supplied by small arteries coming from the submucous tissue, and pouring their blood into corresponding veins, and with this reticular tissue lymphatics are connected...."

"Besides these glands properly so-called, that is to say involutions of the epithelial (hypoblastic) mucous membrane, there are found in the mucous membrane bodies belonging to the lymphatic system also often called glands, viz. the solitary glands and the agminated glands or glands of Peyer."

The glands of Brunner.

"Immediately below the pylorus in man, but varying somewhat in position in different animals, are the glands of Brunner. These may be regarded as modifications of the pyloric glands of the stomach[1]. In each gland a duct, lined with short columnar epithelium cells leaving a distinct lumen, extends single for some distance, and piercing the muscularis mucosæ divides in the submucous tissue into a number of tubes, which subdividing take a twisted course and end in slight enlargements or alveoli. The cells lining both the branching tubes and the alveoli are short cubical cells with an indistinct outline, similar to but, in a fresh condition, more distinctly granular than the cells of the gastric pyloric glands. Bundles of plain muscular fibres, stragglers from the muscularis mucosæ, are scattered among the tubes."

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[1] Heidenhain, *Archiv f. microscop. Anat.* Vol. VIII. (1872), p. 279.

"These glands of Brünner when traced back to the stomach are found to pass gradually into the pyloric glands; traced along the intestine they soon disappear. The ducts of those glands which reach into the duodenum so far as to be found in company with the glands of Lieberkühn and the villi, open into the lumina of the former. It is not clear that any special purpose is served by these glands of Brunner; an extract of the glands is said[1] to digest fibrin in the presence of an acid[2]."

The Villi. "The villi vary in size and form in different animals and in different parts of the intestine of the same animal; each villus, moreover, varies in form at different times; they may be generally described as having the shape of a flattened finger, but are frequently broader at the free end than at the base; they have, in man, a length of about 1 mm. and a breadth of from 0·2 mm. to ·5 mm.[3]" The villi are, like the mucous membrane in general, composed of lymphoid connective tissue covered with a columnar epithelium of somewhat peculiar character, amongst which goblet cells occur, resting on a basement membrane composed of flattened cells. Between the bases of the columnar cells are seen 'cells with a relatively small quantity of cell-substance round the nucleus; these have been taken to be reserve or replacement cells,'[4] and in addition to these numerous lymph corpuscles.

Each villus contains a central lymphatic vessel, or lacteal, which at its upper end is club-shaped and at its base communicates with the lymphatics of the mucous membrane. External to the central lymphatic are fine bundles of involuntary muscular fibres, which are continuous with those of the muscularis mucosæ, and the contraction of which will necessarily tend to compress the contents of the lacteal. Still more external, *i.e.* between the layer of muscular fibres and the basement membrane, are a small artery and vein (conducting blood to and from the villus and communicating with larger vessels in the submucous tissue) and a plexus of capillaries establishing a communication between them. The space between the basement membrane and the central lacteal is occupied by adenoid tissue, *i.e.* by a retiform or reticular connective tissue, the meshes of which are occupied by leucocytes.

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[1] P. Grützner, Pflüger's *Archiv*, Vol. XII. (1876), p. 290.

[2] Foster, *op. cit.* p. 449.

[3] Foster, *op. cit.* p. 445.

[4] *Ibid.* p. 447.

SECT. 2. THE RETIFORM OR RETICULAR (ADENOID) CONNECTIVE TISSUE OF THE INTESTINAL MUCOUS MEMBRANE.

The various structures which occupy the mucous membrane of the intestine are imbedded in, and are supported by, a framework of the particular variety of connective tissue which is known as retiform or reticular tissue—a tissue which serves a similar function in the lymphatic glands and some other organs. "It is composed of a very fine network or reticulum of connective-tissue fibres, which in their behaviour to staining reagents and in their general microscopic appearance closely resemble the white fibres of areolar tissue[1]."

It was, until lately, believed that the network of fibres of reticular tissue are originally formed by the union of the processes of connective tissue cells, and as a result of transformations of the protoplasm of these. This view is probably incorrect. The fixed cells of the tissue, which were supposed to be the centres from which the anastomosing fibres took their origin, are now believed to be merely "applied to and wrapped round the strands of the network, which may thus be in great measure concealed by the cells. The tissue then appears formed of a network of branching and anastomosing cells, and was for a long time so described, but if the cells are brushed away or otherwise removed, as by a short treatment with dilute alkali, the fibres of the reticulum come clearly into view. The true structure of the tissue was first pointed out by Bizzozero[2]." The fibres of retiform connective tissue are probably in no sense developed from the protoplasm of connective tissue cells, but are produced by changes occurring in the original ground substance.

"The view which supposes that a direct conversion of the protoplasm of the connective tissue cells takes place into fibres, both white and elastic, has of late years been widely adopted, but it seems to rest largely upon a desire to interpret the fact in accordance with the conception (originally formulated by Beale and M. Schultze), according to which every part of an organised body consists either of protoplasm (formative matter), or of material which has been protoplasm (formed material); the idea of a deposition or change occurring outside the cells in the intercellular substance being excluded. It is, however, not difficult to shew that a formation of fibres may occur in the animal organism without a direct transformation of protoplasm, although the materials for such formation may be furnished by cells. Thus in those cœlenterates in which a low form of connective tissue first makes its appearance this is distinguished by a total absence of cellular elements, a ground-substance only being developed and fibres becoming formed in it. Again, the fibres of the shell-membrane of the bird's egg are certainly not formed by the direct conversion of the protoplasm of the cells which line the oviduct, although they are formed in matter secreted by those cells, and it is through their agency that the deposit occurs in a fibrous form" (E. A. Schäfer[3]).

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[1] E. A. Schäfer, 'General Anatomy and Histology,' Part II. of Vol. 1 (see p. 239) of Quain's *Anatomy*, London, Longmans, Green and Co., 1891.

[2] E. A. Schäfer, *op. cit.* p. 240.

[3] E. A. Schäfer, *op. cit.* p. 242.

In lymphatic glands, as well as in the mucous membrane of the intestine, the meshes of reticular tissue contain cells which closely resemble the colourless cells of the blood and of the lymph. These are denominated lymphoid cells and the term adenoid or lymphoid connective tissue is applied to the retiform or reticular tissue in the above situations.

Mall's researches.

In the course of his researches on the structure of reticular or retiform tissue, F. P. Mall was led to the conclusion, lately abundantly justified, that the fibres are not identical in chemical composition with the white fibres of areolar tissue. He was at first inclined[1] to look upon them as composed of a substance closely resembling elastin, though he subsequently arrived at the correct conclusion that the substance is neither identical with that of white nor of yellow elastic fibres[2]. F. Mall having subjected reticular tissue to the action of boiling water for long periods, found that the aqueous extracts did not gelatinise. He discovered, however, the fact that reticular tissue is not affected by long digestion with trypsin.

R. A. Young's researches.

Under the direction of Professor Halliburton, Young re-investigated the question as to whether reticular tissue is identical with, or differs from, white fibrous tissue. He shewed that Mall had been led into error in supposing that when reticular tissue is boiled, *no* gelatin is formed, but he unfortunately allowed himself to arrive at a conclusion which was not warranted by the facts discovered and which tended to obscure the perfectly correct opinion which Mall had formed as to the individuality of reticular tissue.

Young summarised his researches as follows: "The general outcome of this research is that retiform tissue as contained in the alimentary mucous membrane and lymphatic glands yields a small amount of gelatin, which is however capable of being estimated quantitatively. There are therefore no grounds for supposing with Mall that the fibres which compose the reticulum are different from the white fibres in other connective tissues. Microscopically they have the same characteristics, and the present research shows that they are also the same in chemical composition[3]."

Siegfried's researches.

In a dissertation published at the close of the year 1892[4], Siegfried shewed that the purified reticular tissue of the intestine is, as Mall had supposed, entirely distinct in chemical composition from white fibrous tissue. When boiled in water for a period of only 20 minutes it splits up, with the formation of a powdery, phosphorus-containing proteid, reticulin, a small quantity of gelatin being formed at the same time. Reticular tissue thus appears to be formed, either of a microscopically undistinguishable mixture of reticulin and collagen (a view which, in addition to its inherent improbability, is disproved by the fact that collagen does not, like reticular tissue, yield gelatin when merely boiled for 20 minutes), or of a substance which when boiled with water splits up into reticulin and gelatin. It is thus as different from white fibrous tissue as it is from elastin.

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[1] F. P. Mall, 'Die Blut- und Lymphwege im Dünndarm des Hundes,' *Abhandl. d. math.-phys. Cl. d. königl. sächs. Ges. d. Wiss.* Vol. xiv. (1887), No. iii.

[2] F. P. Mall, 'Das reticulirte Gewebe,' *Ibid.* Vol. xvii. (1891), No. xiv.

[3] R. A. Young, B.Sc., 'The Fibres of Retiform Tissue,' (from the Physiological Laboratory, King's College, London), *Journal of Physiology*, Vol. xiii. (1892), pp. 332—334.

[4] Dr Max Siegfried, 'Ueber die chemischen Eigenschaften des reticulirten Gewebes' (Habilitationsschrift, &c.), Leipzig, Dec. 1892.

Reticulin.

Preparation of purified retiform tissue from the intestinal mucous membrane.

The mucous membrane of the pig's intestine is employed for the preparation of pure reticular tissue, in preference to that of other animals, because of its comparative freedom from yellow elastic and white fibrous tissue. A separation of the mucous membrane from the submucosa is very readily effected. The raw mucous membrane (Siegfried employed each time that obtained from the intestines of from 8 to 17 pigs) is broken up and washed in large quantities of water, during which process any accidentally adhering fragments of submucous tissue are further separated. Then it is digested at a temperature of 37° C. with a solution of trypsin containing $NaHCO_3$ and Na_2CO_3[1], thymol or chloroform being employed to prevent decomposition. By this process of digestion, the lymphoid cells are got rid of, their protoplasm being dissolved, whilst their nuclei are partly dissolved by the alkaline solution and partly remain suspended in it. At the end of forty-eight hours, the tissue which has been subjected to this process is poured into open vessels and is stirred and kneaded with large quantities of water which are frequently renewed. In order to separate it from water, without contamination with accidental impurities, the tissue is centrifugalised and then repeatedly treated with alcohol and again centrifugalised. The tissue, which has been thus freed from water, is placed in large Soxhlet fat-extracting apparatuses and digested with ether for several days. Having been thus freed from fat, it is again digested during a period of 48 hours with a more concentrated trypsin solution than was employed the first time. The washing with water, alcohol, and ether is repeated as before, when the reticular tissue is obtained in strands of a light-grey colour, which swell up in water, forming delicate porous membranes possessing the structure of the original mucous membrane. When examined microscopically, these membranes appear to be composed of pure reticular tissue, free from the white fibres of connective tissue and from lymph cells.

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[1] Siegfried employed 25 to 30 grms. of the so-called (very active) *pancreatin* made by Parke, Davis and Co. of Detroit, U.S.A., 50 grms. of $NaHCO_3$, *some* Na_2CO_3 and 40 litres of water.

Preparation of reticulin.

When purified reticular tissue is boiled with water for 30 minutes, or even only for 15 minutes, it loses its structure and becomes converted into a light powdery body. If the liquid be filtered and the clear filtrate concentrated and then cooled it gelatinises. The solution answers to all the tests for gelatin, of which the quantity formed is, however, very small.

The powdery body above referred to, having been repeatedly boiled with water, is thoroughly washed, first with water, afterwards with alcohol, lastly with ether; it is then dried, either at 110° C. or at the temperature of the air over sulphuric acid.

Physical and chemical properties of reticulin.

Reticulin is insoluble in water, alcohol, ether, concentrated salt-solutions, lime water, solution of sodium carbonate, and dilute mineral acids. Dilute solution of caustic soda at ordinary temperatures requires weeks to dissolve it. Reticulin gives the biuret and the xanthoproteic reaction, but not Millon's reaction; though it is difficult to obtain a preparation so pure that when treated with Millon's reagent it does not assume a feeble red colour. When only once digested with pancreatin, it always shews this reaction; hence the reason for twice repeating the process, as directed previously. When reticulin is boiled with glacial acetic acid, it partly dissolves and the solution exhibits Adamkiewicz's reaction.

Reticulin is an organic compound containing carbon, hydrogen, nitrogen, sulphur, phosphorus and oxygen; it cannot be obtained free from ash. The latter contains sodium, but no potassium, small quantities of calcium and magnesium, phosphoric acid, chlorine and sulphuric acid.

The following exhibits the mean of the analyses made by Siegfried:—

Carbon	52·88
Hydrogen	6·97
Nitrogen	15·63
Sulphur	1·88
Phosphorus	0·34
Mineral matters	2·27

Reticulin is, as will be seen from the above data, characterised by a high percentage of sulphur. When heated, even for many days, with water at a temperature of 140°, it gives off no sulphuretted hydrogen. The sulphur is, however, in part at least, separated when reticulin is boiled with strong solution of caustic soda or with strong hydrochloric acid.

The phosphorus, which reticulin contains, is in organic combination, as proved by the following fact. When 1 gramme of reticulin is digested with constant shaking at a temperature of 25° C. with dilute nitric acid (containing 4·1 °/$_0$ of $NO_2 . OH$), the solution, when tested with ammonium molybdate, is found to contain no phosphoric acid, even though it has been previously boiled with concentrated nitric acid. Were the phosphorus of reticulin dependent on admixture with nuclein, dilute nitric acid would extract from it metaphosphoric acid, and after boiling with nitric acid the reactions of tribasic phosphoric acid would be obtained.

When heated with diluted solutions of the caustic alkalies reticulin yields a phosphorus-free proteid body and a phosphorus-containing organic compound, which is soluble in chloroform and alcohol, but insoluble in ether or water; it is therefore clear that the phosphorus of reticulin is not due to a nuclein residue.

Products of the decomposition of reticulin when decomposed with hydrochloric acid.

As already stated (note 5, p. 244) reticulin when subjected to long boiling with stannous chloride and hydrochloric acid yields as chief products of decomposition, amido-valerianic acid, besides sulphuretted hydrogen, ammonia, lysine and lysatinine, *but neither leucine nor tyrosine.*

SECT. 3. THE INTESTINAL JUICE OR SUCCUS ENTERICUS.

By the name of intestinal juice, or *succus entericus*, we designate the liquid which is secreted by the glands found in the mucous membrane of the small intestine. Although we are now in possession of ingenious methods which enable us to make valuable observations on the processes of intestinal digestion, the difficulties of obtaining sufficient quantities of intestinal juice, *under perfectly normal conditions*, are so great as to lead us to attach very little value to the published quantitative analyses of the secretion. Moreover, it seems likely that the intestinal juice is not absolutely identical in all sections of the intestinal tract.

In the duodenum, for instance, the secretion of the glands of Lieberkühn is mixed with that of the glands of Brünner, the function of which is but imperfectly understood. In some animals, these glands appear to have the structure and the functions of the pyloric glands of the stomach; in others they probably are rather analogous to the pancreas. But leaving the duodenum out of the question, we are already in possession of facts which indicate that the amount of secretion and its chemical activities are by no means identical in the upper and the lower reaches (if we may use the expression) of the intestinal canal. These facts will be referred to in the sequel.

The Methods of obtaining Intestinal Juice.

The earlier workers either made observations on the contents of the small intestines of animals which had for a long time been deprived of food (Tiedemann and Gmelin[1], Bidder and Schmidt[2]), or isolated loops of intestine in living animals by ligatures and, having replaced them in the abdominal cavity, for a period of 4—6 hours, examined the fluid which had accumulated and which they took to represent the normal intestinal secretion (Frerichs)[3]. If we except the observations made on some cases of intestinal fistula in man, especially those of Busch and Demant, our knowledge of the intestinal juice has been obtained almost entirely by the aid of the experimental methods devised by Thiry and by Vella.

Thiry's fistula.

Thiry[4] conceived the remarkably ingenious idea of isolating a portion from the rest of the small intestine, and establishing a fistulous aperture between the isolated piece and the abdominal wall. An incision having been made in the *linea alba* of a fasting dog, and the abdominal cavity having been opened, a coil of intestine is drawn out, and is carefully cut across at points 10 to 15 cms. distant from one another, care being taken that no large vessels pass between the intestine and the mesentery where the incisions are made. Avoiding injury to the mesentery, the upper and the lower ends of the intestinal tube, from which the above segment has been eliminated, are now most carefully united by interrupted sutures, the continuity of the alimentary canal being thus re-established. The second part of the operation now commences. One end of the resected intestine is closed by a series of sutures; the other end is then stitched to the upper or the lower end of the incision in the abdominal wall. Thus is established a *cul de sac* communicating with the exterior of the body, and the walls of which are composed of intestinal mucous membrane. Although Thiry's operations were performed without antiseptic precautions, recovery occurred in several cases. Under our present conditions, if performed by a competent operator, the operation would almost invariably result in the recovery of the animal.

Vella's double fistula.

Thiry's original operation has been abandoned in favour of an ingenious modification first devised and carried out by Vella[5]. As the procedure is one of great practical importance to the physiologist and the pharmacologist, the Author thinks it useful to describe the operation in detail and to mention modifications which experience has shewn to be necessary and to lead to almost invariable success.

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[1] Tiedemann and Gmelin, *Die Verdauung nach Versuchen*, Vol. I. pp. 102—103 and pp. 154—162.

[2] Bidder and Schmidt, *Die Verdauungssäfte und der Stoffwechsel*, Mitau und Leipzig, 1852, p. 260 *et seq.*

[3] Frerichs, article 'Verdauung' in Wagner's *Handwörterbuch d. Physiologie*, Vol. III. p. 851.

[4] Thiry, 'Ueber eine neue Methode, den Dünndarm zu isolieren.' *Sitzungsber. d. Wiener Akademie*, Vol. L. (1864), p. 77.

[5] Ludwig Vella, 'Ein neues Verfahren zur Gewinnung reinen Darmsaftes und zur Feststellung seiner physiologischen Eigenschaften,' Moleschott's *Untersuchungen*, Vol. XIII. (1881), p. 40; 'Nouvelle méthode pour obtenir le suc entérique pur et pour en fixer les propriétés physiologiques (Résumé),' *Archives Italiennes de Biologie*, Vol. I. (1881), p. 228. The first paper, which the Author has not seen, was entitled 'Nuovo metodo per avere il succo enterico puro e stabilirne le proprietà fisiologiche,' *Memorie dell' Accademia delle Scienze dell' Istituto di Bologna*, Ser. 4, Tom. II. Fasc. 3°.

The abdominal wall of a fasting dog (preferably a bitch) is shaved and thoroughly disinfected by repeated washing with soap and water and then scrubbing with a 1 per mille solution of corrosive sublimate. The instruments to be employed are sterilised and the operator and assistants observe the strictest rules of antiseptic surgery. The animal having been deeply anæsthetised, by means of morphia and chloroform, an incision, about 5 centimetres long, is made through the abdominal wall, in the linea alba. A thoroughly sterilised piece of lint, or linen, is then wrung out of a sterilised solution containing 0·6 per cent. of NaCl and 5 per cent. of carbolic acid, at a temperature of 40° C., and this is placed over the abdominal wall of the dog. The operator then proceeds to select the part of the intestine where the fistula is to be established. Drawing out a loop including the selected portion, he protects it from atmospheric impurities and, so far as possible, prevents too great a lowering of temperature, by covering it with cotton or linen cloths wrung out of the warm antiseptic solution. The portion of intestine to be isolated may be between 200 and 500 millimetres in length, though the difficulties are much greater in the latter than the former case. The first stage of the operation is performed as described previously (Thiry's fistula). The second stage is, however, different and presents greater difficulties. Instead of closing, by sutures, one end of the isolated portion of intestine, and establishing a *cul de sac*, its two ends are separately sewn to the incision in the *muscular wall of the abdomen*, one in front of the other. Two openings are thus established, one in front of the other, of which one communicates with the proximal and the other with the distal portion of the isolated intestinal tube.

The operation of fixing the ends of the intestine to the incision in the abdominal wall is facilitated by previously passing long ligatures through either end. These serve to pull upon, and to hold, the ends in any required position and to prevent their slipping back into the abdominal cavity during the operation. It is highly advisable, also, before proceeding to fix the two ends, *i.e.* as the very first step in the second stage of the operation, to make a longitudinal incision about two centimetres in length through the walls of each end of the gut and then to pass sutures through the peritoneal coat a little distance on either side of this incision. By tying these, the lumen of the gut is narrowed at both ends, the object being to prevent prolapsus of the intestinal mucous membrane, an event which otherwise invariably occurs both in the case of Thiry's and Vella's fistulæ, at periods varying between two or three months from the operation[1].

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[1] At the present time the Author is experimenting on a dog in which a Vella's fistula was established on Jan. 5, 1893, the precaution of narrowing the gut at its

The following details must be remembered in fixing the ends of the intestine to the incision. On no account must the sutures pass through the free edge of the two ends of the gut; they should on the contrary pass through the peritoneal wall of the gut about 8 mm. from the free edge and then through the edges of the muscular wound. In this way the two free ends of the gut project a little above the bottom of the wound and after the process of healing is complete two perfectly accessible fistulous apertures are established. For two or three days after the operation dogs which have been subjected to the operative procedure above described should only be supplied with water. About the third day they may be placed on a milk diet, which should be continued, at least, until the bowels have been moved. If the dog be then apparently normal it may be placed on an ordinary diet. To conclude the description of the operation and the treatment of the dog it may be added that the one essential to success, which is almost invariable[1], is the performance of the operation under the strictest antiseptic precautions; when completed, however, no dressings should be applied to the wound. In the course of about a week it will be nearly, if not completely, healed and observations may be commenced.

The Secretion of Intestinal Juice and the Conditions which influence it.

Although the statements of authors are not absolutely concordant, nearly all who have made observations, either on pathological fistulæ in man, or on dogs with fistulæ established either by the methods of Thiry or of Vella, agree in saying that in the absence of chemical, mechanical, or electrical, stimuli, either no secretion is poured out by the intestinal mucous membrane or, at most, a very small amount.

W. Busch[2], who studied the secretion of the intestinal juice in a woman with a fistula of the upper part of the small intestine, found that in the absence of stimuli, so little fluid was secreted that it was not able thoroughly to moisten a small piece of litmus paper inserted into the gut. Demant[3], on the other hand, who made observations on an intestinal fistula in a man (*affecting, it would appear, the lower part of the small intestine*), observed a very scanty secretion, in the

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extremities in the manner above described having been taken. The success of the procedure has been absolute, not the slightest prolapsus of the mucous membrane having occurred (July 29, 1893).

[1] How great is the influence of antiseptic methods on the success of the experimental physiologist is proved by the fact that whereas if they are adopted death as a result of establishing a Vella fistula is very rare, without them the mortality is very high, 12 out of 18 dogs operated upon by Vella having died from the immediate results of the operation.

[2] Prof. W. Busch, 'Beitrag zur Physiologie d. Verdauungsorgane,' Virchow's *Archiv*, Vol. XIV. (1858), pp. 140—186.

[3] B. Demant, Ueber die Wirkung des menschlichen Darmsafts. Virchow's *Archiv*, Vol. LXXV. (1879), pp. 419—430.

absence of stimuli and in the fasting condition, but the secretion became much more abundant after food. Thiry[1], Quincke[2], Masloff[3], Gumilewski[4], Vella[5], all substantially agree in stating that in the absence of stimuli the secretion of the intestinal juice is either in abeyance or at most very scanty. Whether the secretion be completely absent or only scanty, during the resting and fasting conditions, there can be no doubt that after food has been taken secretion occurs, though not immediately, the amount probably increasing up to the 6th or 7th hour of digestion[6]. We are not as yet in possession of data as to the amount of secretion poured out by different segments of the intestinal mucous membrane during the digestive act. The difficulties of the investigation are remarkably great and, indeed, appear almost insuperable. It is obvious that the secretion poured out, during digestion, by an isolated portion of the intestine which is not affected by the normal stimulus afforded by the intestinal contents can only imperfectly, if at all, quantitatively represent the process going on in the rest of the intestine.

Röhmann, from comparative observations on three dogs with Vella-fistulæ, as well as from an examination of the data of previous observers, has established a strong presumption in favour of the view that the secretion of intestinal juice is much more abundant in the lower than the upper part of the small intestine[7].

Effects of mechanical and electrical stimulation of the mucous membrane.

The mere introduction of a catheter or pieces of sponge into the gut, through a Vella-fistula, is sufficient to provoke a secretion of the intestinal juice. The mucous membrane forming the edges of the fistula becomes injected and from time to time small drops of liquid, mixed at first with masses of mucus, rich in exuviated epithelium, exude. The amount and character of the mucus depend, in no small degree, upon the intensity of the stimulant, which very readily gives rise to a pathological reaction.

Thiry, by mechanical stimulation of a fistula, obtained from a surface of intestine estimated at 30 square centimetres, secretion at the rate of 4 grms. per hour. Other observers obtained smaller amounts; but the data possess no value, seeing that the stimuli were of unknown intensity and provoked a secretion which was probably abnormal in amount, and certainly abnormal in character.

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[1] Thiry, 'Ueber eine neue Methode, &c.' (see p. 406).

[2] Quincke, 'Ueber die Ausscheidung von Arzneistoffen durch die Darmschleimhaut,' *Archiv für Anat. u. Physiol.* 1868, pp. 150—164.

[3] A. Masloff, 'Zur Dünndarmverdauung,' *Untersuchungen a. d. physiol. Institute zu Heidelberg*, II. (1882), pp. 290—306, see p. 300.

[4] Gumilewski, Pflüger's *Archiv*, Vol. XXXIX. (1886), p. 556.

[5] Vella, *op. cit.* (see references, p. 406).

[6] Heidenhain, 'Physiologie d. Absonderungsvorgänge,' Hermann's *Handbuch*, Vol. v. I. p. 170.

[7] Dr F. Röhmann, 'Ueber Secretion und Resorption im Dünndarm' (Aus d. physiol. Institut zu Breslau), Pflüger's *Archiv*, Vol. XLI. (1887), pp. 411—462.

Electrical stimulation of the mucous membrane powerfully excites the flow of intestinal juice (Thiry), but here again it is obvious that we have no guarantee that the liquid secreted preserves its normal characters.

Effects of chemical stimulation of the mucous membrane.

Leuret and Lassaigne applying acetic acid to the intestinal mucous membrane observed secretion to follow[1]. The researches of F. Röhmann have proved very conclusively that solutions of starch, sugars and peptones into the intestine provoke the secretion of intestinal juice, and these observations the Author can independently confirm.

Effects of pilocarpin on the intestinal secretion.

By injecting pilocarpin into the blood, Vella, and afterwards Masloff, obtained a tolerably abundant flow of apparently normal intestinal juice. In a dog with a fistula, Vella obtained, under the influence of pilocarpin, 14 c.c. of intestinal juice in 35 minutes, and on another occasion 18 grms. in one hour. Masloff, after an injection of 0·01 grm. of hydrochlorate of pilocarpin, obtained from one fistula 40 c.c. of succus entericus in 2 hours. That the result is one which is not obtained under all conditions is shewn by an experiment made by the Author. To a dog, with a Vella fistula of the upper part of the jejunum, but which had been fasting for 24 hours, he administered 0·07 grm. of hydrochlorate of pilocarpin by subcutaneous injection, and although abundant salivation with flow of the nasal and lachrymal secretions occurred, no secretion whatever of intestinal juice followed. The anomalous result in this case may have been due either to the fact that the portion of the intestine experimented upon was situated at the very commencement of the jejunum, whilst in Vella's and Masloff's cases it was probably low down, or that in the fasting condition, the intestine is unable to secrete intestinal juice, even under the influence of pilocarpine[2].

Influence of nervous system on intestinal secretion. Moreau's experiment. Paralytic secretion.

Thiry found that stimulation of the vagi had no effect on the secretion of intestinal juice. Budge[3] observed after extirpation of the cœliac and mesenteric plexuses an increase of the secretion. His observations were confirmed by Lamansky[4], though Adrian[5] obtained negative results in the case of dogs. Subsequently,

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[1] Leuret et Lassaigne, *Recherches physiologiques et chimiques pour servir à l'histoire de la digestion*, 1825, p. 141, quoted by Heidenhain, *loc. cit.* p. 171.

[2] The Author has since the experiment above recorded was performed repeated it, the only variation being that the animal was in full digestion. In this case pilocarpin certainly provoked a *visible* secretion, but the amount was so scanty as not to admit of the quantity being determined. This case unquestionably supports Röhmann's belief that the quantity of intestinal juice secreted by the lower part of the small intestine is much greater than that secreted by the upper (Aug. 1893).

[3] Budge, *Verhand. d. k. k. Leopold-Carol. Acad. d. Naturforscher*, Vol. XIX. (1860), p. 250.

[4] Lamansky, *Zeitschr. f. rat. Med.* 1866, p. 59, quoted by Heidenhain.

[5] Adrian, Eckhardt's *Beiträge z. Anat. u. Phys.*, Vol. III. (1863), p. 61.

Budge's statements received confirmation and extension from the researches of Lauder Brunton and Pye Smith[1].

"When all nervous connection between the intestine and the higher nerve centres is cut off, by completely dividing the intestinal nerves, a copious secretion occurs in the intestine. This is best shewn by isolating three loops of intestine, by means of ligatures, after they have been previously carefully emptied, as shewn in fig. 24.

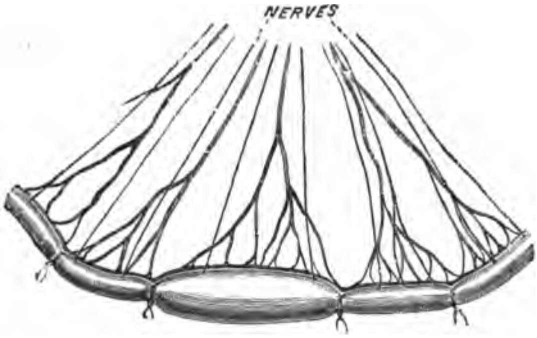

FIG. 24. DIAGRAM SHEWING THE EFFECT OF SECTION OF NERVES ON SECRETION FROM THE INTESTINE. (Brunton.)

The nerves going to the middle loop have been divided, and it is distended with the fluid secreted.

"The nerve-fibres going to the middle loop are then divided, and the intestine is returned to the abdominal cavity. After four or five hours, the animal is killed and the intestine is examined; it is then found that the loop, the nerves of which have been divided, is filled with fluid, while the other loops which have been under precisely the same circumstances, but the nerves of which have not been cut, remain empty" (Moreau's experiment[2]).

"It is evident, then, that certain *nerve-centres* possess the power of *restraining* the *secretion* from the intestine. These nerve-centres have been shewn by Brunton and Pye-Smith to be the smaller or inferior ganglia of the solar plexus, with the superior mesenteric offset from them. When these ganglia are destroyed, the same abundant secretion occurs in the intestine as when all the nerves are cut, but if these ganglia be left intact the spinal cord may be removed, the vagi and splanchnics cut, and the semilunar ganglia excised without any excessive secretion occurring in the intestine[3]."

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[1] T. Lauder Brunton and P. Pye-Smith, 'Intestinal Secretion and Movement,' *British Assoc. Reports*, 1874, 1875, 1876.

[2] A. Moreau, 'De l'influence de la section des nerfs sur la production de liquides intestinaux,' *Comptes Rendus*, Vol. LXVI. (1868), p. 554.

[3] T. Lauder Brunton, *A Text-book of Pharmacology, Therapeutics and Materia Medica*. Third edition. London, Macmillan and Co. 1887 (p. 380).

SECT. 4. THE PHYSICAL AND CHEMICAL CHARACTERS OF THE INTESTINAL JUICE.

The intestinal juice is a pale yellow, usually somewhat turbid, liquid, of powerfully alkaline reaction, possessing, according to Thiry, a density of about 1010.

It is precipitated by alcohol, by nitric acid, and by nitric acid and heat, the precipitate being one of albumin.

When treated with dilute acids, it effervesces, in consequence of the considerable quantity of sodium carbonate which it contains.

In the juice obtained by mechanical irritation, Thiry found from 2·2 to 2·8 per cent. of solid matters, from 0·7 to 1·2 per cent. of albumin, and from 0·7 to 0·9 per cent. of ash, but the result is certainly abnormal. Gumilewski in more recent and more reliable researches on a dog with a Vella fistula situated 1·64 metre from the pylorus and 0·48 m. from the cæcum found the solid matters to amount, on an average, to 1·50 per cent.; these containing 0·43 per cent. of Na_2CO_3 and 0·49 per cent. of NaCl.

From the researches of Gumilewski and Röhmann it would appear that the alkalinity of—in other words the amount of Na_2CO_3 in—the intestinal juice is remarkably constant for the secretion of the same fistula.

The Enzymes of the Intestinal Juice.

In investigating the enzymes of the small intestine three methods of inquiry have been pursued: (1) the mucous membrane of recently killed animals has been extracted with solvents, such as glycerin, which have the property of dissolving enzymes, and the digestive activity of the extracts has been determined in the case of the various groups of alimentary constituents, (v. Wittich, Masloff): (2) the intestinal mucous membrane has been carefully dried in the air and portions of the dried mucous membrane have been digested with the substances under investigation (Brown and Heron); this method has only been applied to the investigation of the enzymes which act upon carbohydrates, but has in the case of these yielded results of the greatest interest and value: (3) the intestinal juice has been collected and its activity determined by artificial digestions carried on in the incubator: (4) the alimentary constituents, either solid or in solution, have been introduced into a segment of intestinal tube in animals with Thiry- or Vella-fistulæ, and the products of digestion have been examined.

Action of the intestinal juice on proteids.

The earlier experimenters (Thiry, Leube[1], Schiff[2]) asserted that the intestinal juice possessed proteolytic properties. Thiry limited this power to the digestion of raw fibrin, whilst Schiff asserted that in the case of successful fistulæ, all proteids are digested and converted into peptones, if introduced within the intestine. Subsequent observers, however, such as v. Wittich[1], Quincke[2], Demant[3], Paschutin[4], using different methods, were unable to confirm the observations of Thiry, Leube and Schiff.

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[1] W. Leube, 'Beiträge zur Kenntniss des Dünndarmsaftes,' *Habilitationsschrift*, Erlangen, 1868; 'Ueber Verdauungsproducte des Dünndarmsaftes,' *Centralblatt f. d. med. Wissensch.* 1868, p. 289.

[2] Schiff, 'Nuove ricerche sul potere digerente del succo enterico,' *Il Morgagni*, 1867, No. 9. Abstracted in *Centralblatt f. d. med. Wiss.* 1868, p. 357.

The most recent researches (Masloff, Wenz) have clearly proved that neither the mucous membrane of the small intestine nor the juice which it secretes, contain enzymes capable of converting proteids into albumoses and peptones, under the conditions which exist in the small intestine.

Masloff[5], in a research conducted in Kühne's laboratory, has shewn that the succus entericus has no action on proteids *provided that the influence of putrefactive organisms be prevented by the addition of thymol.* If acidified, the intestinal juice exerts (according to Masloff) a *very slight* solvent action on fibrin, due to the presence, obviously, of a trace of pepsin, the physiological importance of which is altogether insignificant. Masloff found that proteids of various kinds introduced into a Thiry fistula underwent no change in the course of 24 hours. Wenz[6] has further shewn that the albumoses are not converted into peptones by the intestinal juice.

It is therefore clear that the changes observed to occur by Thiry and Schiff, when fibrin and other proteids were introduced into the intestine of dogs, were the result of a putrefactive process.

Action of the intestinal juice on starch. The diastatic ferment of the intestinal juice.

In spite of the negative statements of many of the earlier observers (Thiry, Leube, Schiff, and v. Wittich)[7], there is now no doubt whatever that the small intestine possesses the power of converting starch into maltose (Brown and Heron[8], Gumilewski[9], Röhmann[10], Dobroslawin[11], Lannois and Lépine[12], &c.). Röhmann's observations

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[1] v. Wittich, *Archiv f. Phys.* Vol. II. p. 193.

[2] H. Quincke, 'Ueber die Ausscheidung von Arzneistoffen durch die Darmschleimhaut,' *Archiv f. Anat. u. Phys.* 1868, p. 150.

[3] B. Demant, *op. cit.* Virchow's *Archiv*, Vol. LXXV. (1879), p. 419.

[4] Victor Paschutin, 'Einige Versuche mit Fermenten, welche Stärke und Rohrzucker in Traubenzucker verwandeln,' *Archiv f. Anat. u. Phys.* 1871, p. 305.

[5] A. Masloff, 'Zur Dünndarmverdauung,' Kühne's *Untersuchungen*, &c. Vol. II. p. 920, Heidelberg, 1882.

[6] Wenz, 'Ueber das Verhalten der Eiweissstoffe bei der Darmverdauung,' *Zeitschrift f. Biologie*, Vol. XXII. 1886, p. 1.

[7] Bidder and Schmidt and Frerichs must be mentioned as early experimenters who attributed a diastatic action to the intestinal juice.

[8] Horace T. Brown and John Heron, 'Die hydrolytischen Wirkungen des Pankreas und des Dünndarms,' *Annalen d. Chemie u. Pharmacie*, Vol. CCIV. (1880), pp. 228—251.

[9] Gumilewski, Pflüger's *Archiv*, Vol. XXXIX. (1886), p. 556.

[10] F. Röhmann, 'Ueber die diastatische Wirkung des Darmsaftes und die Resorption vom Stärkekleister,' *op. cit.* Pflüger's *Archiv*, Vol. XLI. (1887), p. 424.

[11] A. Dobroslawin, 'Beiträge zur Physiologie des Darmsaftes,' *Untersuchung. aus d. Inst. f. Phys. u. Hist. in Graz*, Leipzig, 1870.

[12] Lannois and Lépine, 'Sur la manière différente dont se comportent les parties supérieures et inférieures de l'intestin grêle au point de vue de l'absorption et de la transudation,' *Archives de Physiologie*, 1883, T. I. p. 92.

seem to indicate that the diastatic activity of the intestinal juice secreted in the upper part of the small intestine is very much greater than that of the juice secreted by the ileum. That this activity may play no unimportant part in the intestinal digestion of some animals is rendered probable by an experiment of Röhmann's, in which 50 c.c. of a 2 °/₀ starch mucilage were absorbed by a segment of jejunum 20 centim. long in the course of one hour[1], it being probable that the process of absorption presupposes an antecedent diastatic conversion of starch into sugar.

Brown and Heron have shewn that under the influence of the diastatic ferment of the intestine, starch is converted into maltose, the latter sugar being, however, rapidly converted into dextrose through the agency of another enzyme. Their experiments led them to the opinion that the mucous membrane of the ileum is richer in diastatic ferment than that of the duodenum or jejunum, and in this respect they do not agree with the direct observations of Röhmann on dogs with Vella-fistulæ.

Action of the intestinal juice on cane sugar. The 'intestinal inverting enzyme.'

In the year 1871, Paschutin[2], for the first time, drew attention to the fact that the intestinal mucous membrane, in its whole extent from the pylorus to the ilio-colic valve, contains a ferment which converts cane-sugar into grape-sugar. When cane-sugar is boiled with dilute mineral acids, or subjected to the action of so-called inverting ferments (of which one exists in yeast), it is readily split up into a molecule of dextrose and a molecule of levulose, thus:—

$$\underbrace{C_{12}H_{22}O_{11}}_{\text{Saccharose.}} + H_2O = \underbrace{C_6H_{12}O_6}_{\text{Dextrose.}} + \underbrace{C_6H_{12}O_6}_{\text{Levulose.}}.$$

To the process the term *inversion* is applied, and to the mixed sugar which results from it the name of *invert-sugar*.

The researches of Röhmann[3], Bastianelli[4], and particularly of Brown and Heron[5], have demonstrated in the fullest manner the accuracy of Paschutin's discovery that an inverting enzyme is contained in the intestinal mucous membrane and in the intestinal juice. There is, however, a consensus of opinion on the part of those who have experimented on this subject that the mucous membrane possesses a much more marked inverting action than the intestinal juice itself, so that inversion is obtained much more readily and perfectly if a solution of cane-sugar be allowed to sojourn for a short time in the intestine and thereafter be digested in the incubator, or if it be digested with a small piece of air-dried mucous membrane, than if it be mixed with succus entericus and then placed in the incubator.

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[1] Röhmann, *op. cit.* p. 429.

[2] Dr V. Paschutin, 'Einige Versuche mit Fermenten, welche Stärke und Rohrzucker in Traubenzucker verwandeln,' *Archiv f. Anat. u. Physiol.* 1871, pp. 305—384. Refer specially to p. 374. The merit of this very important discovery has been erroneously attributed by many writers to Claude Bernard. Thus Röhmann says, "Auf die invertierenden Eigenschaften des aus Thiry'schen Fisteln gewonnenen Darmsaftes hat zuerst Cl. Bernard aufmerksam gemacht." "J'ai découvert qu'il possède une action inversive très puissante sur le sucre de canne." Bernard, *Leçons sur le Diabète*, Paris, 1887, p. 259.

[3] Röhmann, *op. cit.* p. 432.

[4] C. Bastianelli, 'Ueber die physiologische Bedeutung des Darmsaftes.' Abstracted from the original paper in the *Bollet. d. R. Accad. med. di Roma*, Vol. XIV. pp. 140—180, in *Jahresb. f. phys. Chem.* 1889, p. 238.

[5] Horace T. Brown and John Heron, 'Die hydrolytischen Wirkungen des Pankreas und des Dünndarms,' *Annalen d. Chemie u. Pharmacie*, Vol. CCIV. (1880), pp. 228—251.

In harmony with our knowledge of other enzymes, we may provisionally, and with considerable probability, assume that the mucous membrane of the intestine contains a zymogen of the inverting ferment which is sparingly diffusible, and that this zymogen is broken up with the liberation of the free ferment when the cells which contain it are in contact with cane-sugar. Brown and Heron have shewn that the inverting action is never complete. As soon as about 25 °/₀ of cane-sugar has been inverted, the process comes to an end. It must be remembered, however, that experiments performed *in vitro* give us but an imperfect conception of what occurs in the living body. We have experimental proof of the facility with which sugars are absorbed by the small intestine, and it is probable that as the absorption of invert-sugar will occur *pari passu* with its production, the conditions are present for the complete inversion of any cane-sugar existing in the intestine.

In Röhmann's experiments, the intestinal juice, obtained from a Vella-fistula situated in the upper half of the small intestine, possessed much greater inverting power than that of two other fistulæ situated in the lower half. Brown and Heron, by digesting weighed quantities of mucous membrane of pig's intestine with 3 per cent. solutions of cane-sugar, at 40° C., found that the duodenum, including Brunner's glands, possesses most feeble inverting power, no inversion having occurred after digestion at this temperature during a period of 3½ hours. The jejunum and ileum possess very much higher inverting activity and in about equal degree, except in the situation of Peyer's patches, where the activity is at its highest.

If we except man in a state of civilisation, cane-sugar can only rarely find its way into the intestine, and it therefore appears strange that an inverting ferment should be so widely distributed throughout the intestinal mucous membrane. In all probability, however, the inverting ferment which can resolve saccharose into dextrose and levulose is the same ferment which possesses the power of splitting up sugar of milk into dextrose and galactose. If this be the case we have a ready explanation of its wide diffusion.

The maltose-converting enzyme.

When malt-diastase acts upon a starch solution, the sugar which results from its action is maltose. When the diastatic ferment of the pancreas acts upon starch, maltose is also formed, though subsequently a small fraction of this maltose is converted into grape sugar, according to Brown and Heron.

We may assume that, in addition to its diastatic enzyme, the pancreas contains a trace of another ferment, capable of splitting-up maltose, though the quantity of this ferment is altogether insufficient to effect the complete conversion. Whilst the observations of Sheridan Lea[1] throw some doubt on the existence of the latter enzyme in the pancreas, the researches of Brown and Heron have, however, led to the interesting discovery that the mucous membrane of the small intestine (doubtless also, though perhaps less, the intestinal juice) possesses, in an intense degree, the power of converting maltose into grape sugar. The activity of the mucous membrane of the small intestine in this respect is said to be much greater as we approach its lower end, the maximum activity being possessed by those parts of the jejunum and ileum in which Peyer's patches are situated.

The part played by the intestinal mucous membrane in reference to the digestion of starches is thus seen to be complementary of that exerted by the diastatic ferments of the saliva and the pancreatic juice. Under the influence of these, the starch of the food is resolved into dextrines and maltose, the amount of the latter rapidly amounting to 80 per cent. of the starch digested. When digested with the intestinal mucous membrane, this maltose is rapidly and completely converted into grape sugar. The inversion of cane sugar by the inverting ferment is, on the other hand, a process which proceeds somewhat slowly, and which, as was previously stated, comes to a standstill (in experiments *in vitro*) after the conversion has affected 25 per cent. of the saccharose present. The conversion of maltose into grape sugar takes place rapidly and is a continuous process, resembling in this respect the action of dilute sulphuric acid, which, with the aid of heat, can effect the complete inversion of cane-sugar.

The action of the intestinal juice on fats.

The intestinal juice possesses no special enzyme which acts upon fats. When intestinal juice is shaken with a neutral fat containing no trace of free acid, no permanent emulsion results. When however, as is always the case after the action of the pancreatic juice upon the neutral fats, a trace of free acid is present, the intestinal juice forms a durable emulsion with fats. This property depends upon the sodium carbonate which the juice contains, and which, as was pointed out, amounts on an average to 0·5 per cent. In reference to its action on fats, indeed, the intestinal juice has been supposed to act as a dilute solution of sodium carbonate, and Bunge has argued strongly in favour of the view that the chief function of the juice is to aid in the emulsionising of fats and thus promoting their subsequent absorption. But such a view appears to the Author a very one-sided one, as the presence of an alkaline fluid on the surface of the intestinal mucous membrane would further not only the absorption of fat but also the progress of proteolysis by trypsin. The capital objection to Bunge's conception of the part played by the intestinal juice is that, as will be subsequently shewn, the alkaline carbonate which it contains is more than neutralised by the lactic acid which is formed by the action of micro-organisms, so that the reaction of the contents of the small intestine is not alkaline, but acid. Whether it plays any part whatever in furthering their absorption, the action of the intestinal juice on fats is, doubtless, insignificant, when compared with the part which the intestinal enzymes and ferments play in the digestion of the carbohydrates.

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[1] Sheridan Lea, 'A Comparative Study of Artificial and Natural Digestion,' *Journ. of Phys.* Vol. XI. (1890), p. 227.

Summary of the actions of the intestinal secretion.

The intestinal mucous membrane and its secretion have thus been shewn to exert no chemical action on the proteid constituents of food, but to play a great part in the digestion of the carbohydrates of the economy, completing, if necessary, the conversion of starch into soluble products, splitting up saccharose and lactose into glucoses, but above all, converting maltose into grape sugar. When we consider the large proportion of carbohydrates which herbivorous animals consume, the great importance of this function to the economy will be manifest.

CHAPTER X.

THE CHEMICAL PROCESSES WHICH HAVE THEIR SEAT IN THE INTESTINES AND WHICH ARE THE RESULTS OF THE ACTIVITIES OF MICRO-ORGANISMS. THE PRODUCTS OF THESE PROCESSES.

Introductory Remarks.

When treating of the influence of changes in the acidity of the gastric juice in disease (p. 167 *et seq.*) its antiseptic action was discussed, and it was shewn that the acid which it contains exerts a marked influence in destroying the putrefactive, as well as many of the pathogenic, organisms which may find their way into the stomach. In the normal condition, gastric digestion in a healthy man is a process which proceeds entirely under the influence of enzymes, and the fermentations which are the results of the activities of organised ferments must be looked upon as a departure from normality—as, indeed, pathological. In the small, but much more so in the large, intestine, the conditions are more favourable to the growth of microbes, and hence, side by side of the digestive processes, properly so-called, others are going on which are due to the intervention of imported germs. In the herbivora, the action of organised ferments attains a high importance, seeing that it is apparently through their action that a part of the cellulose is utilised, which forms so large a part of the food consumed. In man and the carnivora, however, organised ferments play a secondary part in the processes of the small intestine, and digestion appears to be most typically physiological when their activity is least conspicuous, and when certain, at least, of the products of that activity are smallest in amount.

It is customary to speak of the putrefactive processes of the small intestines, but the expression is an inaccurate one. When we open the small intestines immediately after death, we find their contents entirely free from putrefactive odour, properly so called. It is only when we examine the contents of the large intestine that we become aware of the presence of fœtid products, which increase in amount as the contents undergo the changes which ultimately result in their transformation into the fæces. There can be no doubt that special influences must be at work to limit, or rather to modify, the putrefactive processes in the small intestines, and especially to prevent the formation of those fœtid products which are the essence of the putrefactive process. In discussing the disinfecting function of the bile it was argued that this fluid probably exerts a real disinfecting action, as Maly and Emich have contended, in virtue of the bile acids which it contains, though Voit and Röhmann deny this special action.

Macfadyen, Nencki, and Sieber have shewn that, contrary to the general belief, but in accordance with many previous observations by reliable investigators, the contents of the small intestine are invariably acid[1]. The presence of free acid in the small intestine acts doubtless both by setting free the antiseptic bile acids and by the destructive and inhibitory influence which free acids exert on many forms of bacterial life.

Accordingly, the above-named observers have found that in the small intestine of man the products of the putrefactive decomposition of proteids are absent, the organisms which are able to occasion their decomposition being absent. Although, as has been argued, the putrefactive processes in the small intestine are, in the perfectly physiological condition, comparatively insignificant in amount, we must yet consider them in some detail, inasmuch as they play a much more important part in disease, and are, besides, constant products of the processes which occur in the large intestine. Processes of fermentation affecting the carbohydrates, as distinguished from putrefactive processes, normally occur on a considerable scale in the small intestine.

SECT. 1. THE DECOMPOSITION OF THE PROTEIDS UNDER THE INFLUENCE OF BACTERIAL ACTION.

In discussing the action of trypsin on the albuminous bodies we have shewn that, in addition to the albumoses and peptones, there result, from the profoundly decomposing action of that enzyme, many amido-acids of the fatty group, paroxyphenylamidopropionic acid or tyrosine, certain bases (lysine, lysatinine and ammonia), and a chromogen which we designate tryptophan. Although there is evidence that the complex albuminous molecule contains a variety of aromatic groups, it is noteworthy that the only primary aromatic compound which results from the action of trypsin is tyrosine. Under the influence of the putrefactive bacteria, the same products are at first formed. Other bodies, however, rapidly arise, certain of which (aromatic oxy-acids and phenols) are the products of decomposition, reduction, and oxidation, of tyrosine, whilst others (indol, methyl-indol or skatol, skatol-carbonic acid) are aromatic compounds which are not directly related to tyrosine and which represent a specific decomposition of the albuminous molecule distinguishing bacterial from purely trypsin-proteolysis. According to Baumann, neither indol nor skatol is a primary product, but arises from the decomposition of a body soluble in a mixture of alcohol and ether[1].

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[1] A. Macfadyen, M. Nencki and N. Sieber, 'Untersuchungen über die chemischen Vorgänge im menschlichen Dünndarm,' *Archiv f. exp. Path. u. Pharm.* Vol. XXVIII. (1891), p. 311.

In addition to the aromatic compounds, there are formed, amongst the products of the bacterial decomposition of proteids, volatile fatty acids, certain bases, nitrites, hydrogen, and sulphuretted hydrogen.

The following list includes the products of the bacterial decomposition of the albuminous bodies.

Products of the Bacterial Decomposition of the Albuminous Bodies.

(*The bodies marked with an asterisk have not been found in the intestinal contents.*)

BODIES OF THE FATTY SERIES.

Amido-acids.

Fatty acids.

(*Tetra- and penta-methylendiamin*) only found in the contents of the intestines in pathological conditions—cystinuria, cholera and dysenteric diarrhœa.

BODIES OF THE AROMATIC SERIES.

Indol.

Skatol.

Skatol-carbonic acid[2].*

Tyrosin.

Oxyphenylpropionic acid.*

Oxyphenylacetic acid.*

Phenylpropionic acid.*

Phenylacetic acid.*

Parakresol.

Phenol.

End-products:—carbon dioxide, water, ammonia, nitrites, hydrogen, sulphuretted hydrogen.

Bacterial decomposition not dependent on the formation of trypsin.

It was Kühne who first pointed out that indol is a typical product of the decomposition of albuminous bodies when these are subjected to the combined action of trypsin and putrefaction[3], as well as when they are fused with caustic alkalies[4], but that this body is never produced by the action of trypsin, however long that action may continue. He further shewed that this enzyme is not formed by, and does not exist in connection with, bacterial putrefaction[5].

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[1] Baumann, *Ber. d. deutsch. chem. Gesellsch.* Vol. XIII. p. 284.

[2] Zumft, in a recent research made in Nencki's laboratory at St Petersburg has found indol, skatol, phenol and parakresol in the contents of the large intestine of man, but no skatol-carbonic acid. In his paper entitled 'Sur les processus de putréfaction dans le gros intestin de l'homme, &c.,' *Archives des Sciences Biologiques*, &c. Tome I. pp. 497—517. St Petersburg, 1892.

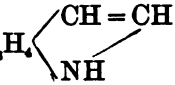
[3] W. Kühne, Virchow's *Archiv*, Vol. XXXIX. p. 165.

[4] W. Kühne, 'Ueber Indol aus Eiweiss,' *Ber. d. deutsch. chem. Gesell.* Vol. VIII. (1875), p. 206.

[5] W. Kühne, 'Erfahrungen und Bemerkungen über Enzyme und Fermente,' *Untersuch. a. d. phys. Inst. Heidelberg*, Vol. I. (1878), pp. 291—324.

INDOL, SKATOL AND SKATOL-CARBONIC ACID.

1. *Indol*, C_8H_7N,



$$C_6H_4\begin{cases} CH = CH \\ NH \end{cases}$$

Indol was first discovered by Baeyer in the products obtained by distilling oxyindol with zinc-dust[1]. It was afterwards found by Nencki[2] and by Kühne[3] amongst the products of the putrefaction of proteids, as well as in those obtained when the proteids are fused with caustic alkalies. Indol, together with skatol (which almost invariably accompanies it), is found in the contents of the large intestine and in the fæces.

Mode of preparation of indol and skatol (Salkowski's process).

Two kg. of well-pressed fibrin are placed in a roomy flask (of the capacity of 12 litres), together with 8 litres of water in which 2 grms. of KH_2PO_4 and 1 gr. of $MgSO_4$ have been dissolved; to this fluid are added 200 c.c. of a saturated solution of Na_2CO_3 and then some cubic centimetres of a putrefying flesh infusion together with fragments of decomposing meat. The flask is then closed with a stopper, which has been bored and provided with a glass tube; to the latter is attached an indiarubber tube connected with a wash bottle half filled with water. The indiarubber tube is provided with a clamp, which is left open during the first days of the experiment. The mixture is digested at a temperature of 40°—42° C. for a period of 5 or 6 days, the flask being shaken from time to time. As soon as the evolution of gas has ceased, the clamp, above referred to, is closed, and only opened from time to time to liberate the gases which have accumulated.

At the end of the time mentioned, the fluid contents of the flask are distilled off, until the residue in the flask measures only 1 to 1·5 litres. The strongly ammoniacal distillate having been acidulated with hydrochloric acid, which decomposes the sulphide of ammonium present, is then precipitated with solution of sulphate of copper and filtered. The clear filtrate is thoroughly shaken up, in repeated fractions, with ether in a stoppered separating funnel of about half-litre capacity. The united ethereal extracts are distilled until the residue measures 500 cc., the residue is twice thoroughly shaken with solution of caustic soda, with the object of separating phenol and traces of acids. The ether is then distilled off at the lowest possible temperature, and the oily residue having been treated with caustic soda, is distilled in a current of steam, until no more indol distils over. The distillate is again shaken with ether, the ethereal solution distilled at the lowest possible temperature, and the residue is evaporated in a deep flask until, on being allowed to cool, the residue sets as a crystalline mass. The latter is then dried in an exsiccator over sulphuric acid. It may be purified from skatol by recrystallising from water. The yield of indol, by the process described, amounts to about 6·5 parts per 1000 parts of water-free fibrin, or, using the quantity of pressed, but yet moist, fibrin recommended, the amount obtained is about 3 grammes.

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[1] Baeyer, *Ann. Chem. u. Pharm.* Vol. CXL. p. 295, and A. Emmerling and C. Engler, *Ber. d. deutsch. chem. Gesellsch.* Vol. I. p. 17, and Vol. III. p. 885.

[2] M. Nencki, 'Ueber die Harnfarbstoffe aus der Indigogruppe und über die Pankreasverdauung.' *Ber. d. deutsch. chem. Gesellsch.* Vol. VIII. (1875), p. 336. M. Nencki, 'Ueber das Indol,' *ibid.* Vol. VIII. (1875), p. 722.

[3] Kühne, *Ber. d. deutsch. chem. Gesellsch.* Vol. VIII. (1875), p. 206

It was previously stated that, according to Baumann, indol and skatol are not directly split off from the proteid molecule but are products of decomposition of an intermediate substance. E. and H. Salkowski have specially studied this matter in reference to indol and, as a result of their experiments, have arrived at the following conclusion in reference to indol: "That in the putrefaction of albumin, indol is not immediately liberated from the albumin in a free condition, but that an intermediate product is formed, which is gradually decomposed by the further action of bacteria. This intermediate product is still unknown; it is not, however, peptone, the quantity of which is (throughout the process of preparation) small, and in the later stages seems entirely to disappear[1]."

Alleged disappearance of indol as a result of prolonged putrefaction.

According to Odermatt[2], Nencki[3], and Brieger[4], if putrefaction be long continued, the quantity of indol gradually diminishes. Thus, in an experiment which lasted five months, Nencki found that the products of putrefaction contained no indol, but only skatol, whilst Brieger, in the products of putrefaction of liver, found no indol after a lapse of only eleven days. Salkowski[5] has, however, come to the conclusion that indol is not destroyed by the long continuance of putrefaction, and that its non-discovery in the above experiments is to be explained by the fact that they were conducted in open vessels and that the substance had disappeared through evaporation.

Physical and chemical properties of indol.

Indol crystallises from hot aqueous solutions in the form of small scales, the melting point of which is 52° C., and the boiling point 245°—246° C. Indol, however, partly decomposes when heated to its boiling point, so that it is advisable to distil it, as recommended in the directions for its preparation, in a current of steam. The vapour of indol possesses a peculiar, disgusting, fæculent smell. Indol is tolerably soluble in hot, and less soluble in cold, water; it is easily soluble in alcohol, ether, chloroform, benzol, and petroleum ether.

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[1] E. Salkowski, 'Zur Kenntniss der Eiweissfäulniss, 1: Ueber die Bildung des Indols und Skatols, nach gemeinschaftlich mit H. Salkowski in Münster angestellten Versuchen,' *Zeitschrift f. physiol. Chemie*, Vol. VIII. (1883—4), pp. 417—466. Refer to Sect. VII. 'Ueber den Modus der Entstehung des Indols aus dem Eiweiss,' *op. cit.* pp. 454—457.

[2] Odermatt, 'Zur Kenntniss der Phenolbildung bei der Fäulniss der Eiweisskörper,' *Journ. f. prakt. Chemie*, Vol. XVIII. (1878), p. 249.

[3] Nencki, *op. cit. Centralb. f. d. med. Wiss.* 1878, No. 47.

[4] Brieger, 'Ueber die aromatischen Produkte aus Eiweiss,' *Zeitschr. f. phys. Chemie*, Vol. III. (1879), p. 134 *et seq.*; see p. 139.

[5] Salkowski, *op. cit. Zeitsch. f. phys. Chem.* p. 557 *et seq.*

Tests. 1. A watery solution of indol when treated with strong yellow nitric acid, or better still with a 2 per cent. solution of sodium nitrite and pure nitric acid, is either coloured red or furnishes a red precipitate of nitrate of nitroso-indol $C_{16}H_{13}(NO)N_2$, HNO_3, which is very slightly soluble in water, readily soluble in alcohol, and insoluble in ether, and is readily decomposed. If the solution is so dilute as not to yield a precipitate spontaneously, this may be caused by shaking with chloroform, when at the line of junction of the colourless chloroform and the red-coloured liquid a red precipitate becomes visible.

"The characteristic red colouration which cholera cultures in bouillon exhibit when they are treated with dilute sulphuric acid ('*Cholera-reaction*') depends upon the simultaneous production of indol and nitrous acid by the cholera spirillum. The nitrous acid is set free by the dilute sulphuric acid and then acts upon the indol, and the red nitroso-indol is then formed, which is identical with cholera-red. 'Vibrio *Metschnikoffii*' also exhibits the cholera reaction[1]."

"The nitrous acid test for indol enables us readily to detect the body in the products of a pancreatic digestion which has not been aseptic. With this object the fluid resulting from the digestion is distilled, and to every 200 or 300 cc. of the distillate are added from 5—8 cc. of a reddish-yellow nitric acid. The liquid thus treated assumes the colour of arterial blood and in the course of some hours deposits the red precipitate, composed of nitrate of nitroso-indol. By dissolving this precipitate in a little hot absolute alcohol and then adding ether, the substance is obtained in the form of beautiful red, microscopic, needles. The red colouration which pancreatic juice exhibits when treated with impure nitric acid was first observed by Claude Bernard[2]" (see p. 264).

2. A small piece of pine wood moistened with strong hydrochloric acid and then plunged into an alcoholic solution of indol, acquires a cherry-red colour.

3. (Legal's reaction[3]). When sodium nitro-prusside is added to a solution of indol of 1 in 1000, so as to produce a yellowish colouration, and, thereafter, some drops of solution of caustic soda, the liquid instantly becomes of a deep violet-blue. On acidulating with hydrochloric or acetic acid, the colour at once changes to a deep blue, which is destroyed by an excess of acid. The second stage of the reaction is so delicate that in a solution containing only 1 part of indol in 10,000 of water, a deep blue colouration is observed[4]: Hemala has studied the spectroscopic characters of Legal's reaction[5].

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[1] Dr Th. Weyl, *Lehrbuch d. org. Chemie*, Berlin, 1891, p. 452.

[2] Maly, Hermann's *Handbuch*, Vol. v. II. p. 225.

[3] *Breslauer ärtztliche Zeitschrift*, 1884, Nos. 3 and 4, quoted by Salkowski.

[4] Salkowski, *op. cit. Zeitschr. f. phys. Chem.* Vol. VIII. p. 447.

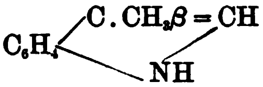
[5] Rich. Hemala, 'Zur Kenntniss der in der chemischen Physiologie zur Anwendung

4. Indol is dissolved in very little benzol, and about three times its weight of crystallised picric acid is then added. The liquid being cautiously heated, enough benzol is added to dissolve the whole. On cooling, the liquid is converted into a magma of red crystals, owing to the union of equal molecules of indol and picric acid. This compound, which presents the appearance of long, red, shining crystals is soluble with difficulty in cold benzol or petroleum ether, but it can be easily recrystallised from its solution in the former. In order to separate indol from its picric acid compound, the latter is decomposed with ammonia, and the solution is shaken with petroleum ether, which readily dissolves the liberated indol. On evaporating the solution, indol in the form of fine crystals is left behind[1].

Fate and transformations of indol in the economy.

A part, probably the chief part, of the indol formed in the intestines is excreted in the fæces A part is, however, especially if constipation or obstruction of the bowels exist, absorbed, oxidised, and excreted in the urine as the potassium salt of indoxyl-sulphuric acid or *indican* (see p. 169). From the amount of the latter substance, we can form a judgment as to the extent of the putrefactive decomposition of proteids in the alimentary canal. The matter will be again considered in discussing the constituents of the urine.

2. *Skatol*[2], C_9H_9N or



$$C_6H_4\begin{cases} C \cdot CH_3\beta = CH \\ NH \end{cases}$$

Skatol or β-methyl-indol, was separated, in Nencki's laboratory by Brieger[3], from the fæces; Nencki[4] afterwards obtained it from the products of pancreatic putrefaction.

Methods of preparation.

Nencki allowed 2330 grms. of fresh pancreas and 500 grms. of meat, free from fat and well minced, mixed with eight litres of water, to decompose during 5 months, the temperature varying during this period between 3·5° C. and 27·5° C. At this temperature, he found only skatol, and no indol amongst the products.

In order to separate the skatol, he added an excess of acetic acid and distilled. The distillate was acidulated with hydrochloric acid and treated with picric acid, which precipitates skatol, in the form of

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gekommenen Nitroprussidsalzreactionen,' Krukenberg's *Untersuch.* Heft 2, pp. 117—136, see p. 134, Jena, 1888.

[1] Hoppe-Seyler, *Handbuch d. phys. u. path. Chem. Anal.* 6te Aufl. Berlin, 1893, p. 163.

[2] From σκῶρ, gen. σκατός, dung.

[3] L. Brieger, 'Ueber die flüchtigen Bestandtheile der menschlichen Excremente' (aus d. Lab. von Prof. Nencki in Bern), *Ber. d. deutsch. chem. Gesellsch.* Vol. x. (1877), pp. 1027—1032.

[4] M. Nencki, 'Vortheilhafte Darstellung des Skatols,' *Centralblatt f. d. med. Wissenschaft.* No. 47.

a picric acid compound which crystallises in the form of beautiful red needles. This compound was then treated with ammonia and distilled in a current of steam, when the skatol distilled over.

Skatol always accompanies indol.

As was previously stated (page 422), Salkowski found that indol is not destroyed during the course of long-continued putrefaction, as Odermatt, Nencki and Brieger had supposed, its non-discovery in their experiments being probably due to its volatilisation. According to Salkowski, from whatever source prepared, all indol contains skatol and the converse is also true[1].

Skatol and indol can replace each other in the products of putrefaction.

As a result of their inquiries E. and H. Salkowski came to the conclusion '*that skatol and indol can replace each other, seeing that in the albuminous molecule skatol does not form one definite fraction and indol another, but both substances take their origin in a common mother-substance which exists in the proteid; this substance, according to circumstances, yields at one time indol in preponderating quantity, and at another time skatol, so that free skatol may be almost absent*[2]."

When we reflect that the relative quantities of indol and skatol differ greatly in different, apparently identical, experiments we are almost forced to the explanation advanced by Salkowski, viz. that the difference in the product must, *cæteris paribus*, depend upon the difference in the agents which bring about the decomposition, to wit, the bacteria. To support his contention Salkowski[3] calls to our remembrance the experiments of Fitz[4] on two organisms, of which the first, when acting on glycerin, nearly always produces ethyl-alcohol, and the second butyl-alcohol. Similarly we may assume the existence of an indol-producing and of a skatol-producing micro-organism, though no one has as yet succeeded in cultivating them separately and identifying them.

Physical and chemical properties of skatol.

Skatol, like indol, crystallises in the form of platelets, which have a melting point of 95° C. and a boiling point of 265°—266° C. It possesses a loathsome, fæcal, odour. It is more sparingly soluble in water than indol, but, in the presence of steam, distils more readily than indol. It is readily soluble in alcohol, ether, chloroform, and benzol.

It forms a compound with hydrochloric acid which has the composition $(C_9H_9N)_2HCl$; this compound is readily soluble in alcohol, but insoluble in water or ether. With picric acid, as already stated, skatol forms a crystalline compound, analogous, and very similar, to the indol compound. It is, however, distinguished from the latter in that, when treated with caustic soda and distilled, skatol passes unchanged into the distillate, whereas the picric acid compound of indol when similarly treated does not furnish indol, in consequence of the latter being decomposed.

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[1] 'In keinem unserer Versuche wurde Indol vermisst,' Salkowski, *op. cit.* p. 448.

[2] Salkowski, *op. cit.* p. 444.

[3] *Ibid.* p. 442.

[4] Alb. Fitz, 'Ueber Spaltgährungen,' *Ber. d. deutsch. chem. Gesellsch.* Vol. XII. (1879), p. 648.

Characteristic reactions.

Skatol is recognised by its crystalline form, its fæcal odour, and its melting point, and by the following reactions:

1. It does not like indol, which it closely resembles, give a red colouration, or precipitate, with nitric acid, containing nitrous acid, but only a milky-white turbidity, which is perceptible in a solution containing 1 part of skatol in 10,000 of water (Salkowski).

2. It dissolves in concentrated hydrochloric acid, the solution possessing a violet colour.

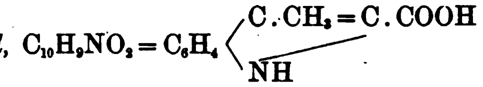
3. When a solution of skatol is treated with a solution of sodium nitro-prusside and then solution of caustic soda is added, an intense yellow colour appears. On now adding one-fourth of its volume of glacial acetic acid and boiling for some minutes the solution gradually becomes violet. The intensity of the colouration, which is not great, increases with time. When shaken with acetic ether, the colouring matter is taken up by it (Salkowski[1]).

4. When skatol is dissolved in benzol and the solution is treated with a solution of picric acid in benzol, the red picric acid compound separates. When distilled with caustic soda, this yields skatol unchanged (*vide supra*).

Fate and transformations of skatol in the economy.

Skatol is in great part excreted in the fæces; some is, however, absorbed, oxidised, and excreted in the urine as one of the so-called ethereal sulphates—skatoxyl-sulphuric acid (compare p. 169).

3. *a-Skatolcarbonic acid*,



$$C_{10}H_9NO_2 = C_6H_4 \left\langle \begin{array}{l} C.CH_3 = C.COOH \\ NH \end{array} \right.$$

In addition to indol and skatol, E. and H. Salkowski discovered, amongst the *constant* products of the putrefaction of proteids, a body which is closely related to the two former, and to which they gave the name of skatolcarbonic acid[2], a name justified by the fact that it may be artificially produced by heating methyl-indol[3] and metallic sodium in a current of CO_2.

Mode of separation.

The method of preparation of skatol-carbonic acid which, unlike indol and skatol, is non-volatile, is so complicated that the reader is referred, for the description, to Salkowski's original paper. The body possesses, however, chemical characters which permit of its ready identification, and the following method suffices to yield a product which enables its reactions to be tried.

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[1] Salkowski, *op. cit. Zeitsch. f. phys. Chemie*, Vol. VIII. (1883—84), p. 448.

[2] E. Salkowski, 'Zur Kenntniss der Eiweissfäulniss, II.: Die Skatolcarbonsäure, nach gemeinschaftlich mit H. Salkowski in Münster i. W. angestellten Versuchen,' *Zeitschr. f. phys. Chemie*, Vol. IX. (1885), pp. 8—33.

[3] Ciamician and Magnanini, 'Ueber die Carbonsäuren der Methylindole,' *Ber. d. deutsch. chem. Gesell.* Vol. XXI. (1888), p. 1925.

Thirty or fifty cc. of the decomposing liquid are concentrated, on the water bath, to about one-fifth their volume, by which means indol and skatol are driven off. The liquid is then acidified with glacial acetic acid and shaken with ether. The ether is separated and shaken with a very weak solution of sodium carbonate, which takes up the acid from the ethereal solution. The sodium skatol-carbonate solution can then be employed for trying the reactions.

Properties of skatol-carbonic acid.

Skatol-carbonic acid occurs in the form of colourless leaflets which are readily soluble in alcohol and ether, and sparingly soluble in water. They melt at a temperature of 164° C. When still further heated, the body splits up into skatol and carbon dioxide.

Reactions.

1. (*Nitric acid and potassium nitrite*[1].) When a solution of the acid (which may only contain 1 part in 1000 of water) is treated with some drops of pure nitric acid (of sp. gr. 1·2) and then with a few drops of potassium nitrite solution (2 °/$_0$), the solution assumes pretty generally a cherry-red colour; it then becomes turbid and deposits a red colouring matter, which is dissolved by acetic ether, when this liquid is shaken up with it. The solution in acetic ether exhibits, if sufficiently diluted, an absorption band in the green. When the solution in acetic ether is shaken up with solution of caustic soda, the former is decolourised, whilst the former acquires an intense yellow colour. If now an excess of hydrochloric acid be added the red colour is restored, and again is dissolved by acetic ether. Instead of acetic ether, amyl-alcohol may be employed, in which the red colouring matter is even more soluble. It is insoluble in ether, benzol, and chloroform. The reaction with nitric and nitrous acids, though reminding one of the indol reaction can be shewn not to depend on the formation of nitrate of nitroso-indol (Salkowski).

2. (Hydrochloric acid and bleaching powder[2].)

The aqueous solution is treated with an equal volume of HCl (of 1·2 sp. gr.) and, afterwards, with some drops of a weak (1—2 °/$_0$) solution of bleaching powder. The mixture gradually acquires a purple-red colour and, after long standing, deposits a purple-red precipitate which is easily soluble in alcohol.

3. (Hydrochloric acid and ferric chloride[2].)

This reaction is much more delicate than the two first. If, to a solution containing 1 part of the acid in 10,000 of water, a few drops of hydrochloric acid be added, and then two or three drops of a very dilute solution of ferric chloride and the mixture be then heated, it becomes, even before the boiling-point is reached, of an intense violet colour. If the solution be more dilute (1:100,000) the reaction is still very distinct; if more concentrated, a larger quantity of acid and iron solution must be added, and the colour is, in that case, an intense cherry-red.

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[1] **Salkowski**, 'Ueber das Verhalten der Skatolcarbonsäure im Organismus,' *Zeitsch. f. phys. Chem.* Vol. IX. (1885), p. 23.

[2] **Salkowski**, *op. cit. Zeitsch. f. phys. Chem.* Vol. IX. p. 25.

Occurrence of skatol-carbonic acid in the urine.

Salkowski has found that when skatol-carbonic acid is introduced into the body, it is excreted unchanged in the urine, where it may readily be detected. He believes that he detected the presence of this body in normal human urine[1]. Baumann does not however hold the evidence on this point to be decisive[2].

DERIVATIVES OF TYROSINE FOUND IN THE PRODUCTS OF THE BACTERIAL DECOMPOSITION OF PROTEIDS.

Besides indol, skatol, and skatol-carbonic acid, certain aromatic bodies which have been shewn to be derived from tyrosine are found amongst the products of the bacterial decomposition of the albuminous and albuminoidal bodies, and therefore in pancreatic digestions complicated with putrefaction. Our knowledge of these aromatic products of putrefaction of tyrosine is based on the researches of Baumann, Brieger, E. and H. Salkowski, Th. Weyl and others. It is to Baumann that we are indebted for the conception of the way in which the principal products of the decomposition of proteids are, probably, related to one another (refer to page 248).

1. According to Baumann, tyrosine, when subjected to putrefaction, yields by a process of reduction, as the first product, hydroparacumaric acid, thus:—

$$C_6H_4\left\{\begin{array}{l}OHp\\ CH_2.CH(NH_2).COOH.\end{array}\right. + H_2$$

Paroxyphenyl-α-amidopropionic acid (Tyrosine)

$$= C_6H_4\left\{\begin{array}{l}OH\\ CH_2.CH_2.COOH.\end{array}\right. + NH_3$$

Para-oxyphenyl-propionic or hydroparacumaric acid.

2. By a process of decomposition and subsequent oxidation (compare equations 2 and 3, p. 248) hydroparacumaric acid yields paroxyphenylacetic acid (E. and H. Salkowski).

$$C_6H_4\left\{\begin{array}{l}OH\\ CH_2.COOH\end{array}\right.$$

Para-oxyphenyl-acetic acid.

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[1] E. Salkowski, 'Ueber das Verhalten der Skatolcarbonsäure im Organismus,' *Zeitsch. f. phys. Chem.* Vol. IX. (1885), p. 32.

[2] Baumann, *Ber. d. deutsch. chem. Gesellsch.* Vol. XIII. p. 284.

3. By a process of decomposition, paroxyphenylacetic acid splits up into carbon dioxide and parakresol, thus (Weyl):

$$C_6H_4\begin{cases}OH\\CH_2.COOH\end{cases} = C_6H_4\begin{cases}OH\\CH_3\end{cases} + CO_2$$

Para-oxyphenyl-acetic acid. **Para-kresol.**

4. By a process of oxidation and subsequent decomposition (refer to equations 5 and 6, p. 248), parakresol yields as products H_2O, CO_2 and $C_6H_5.OH$ or phenol.

In addition to the above products, E. and H. Salkowski have also found phenyl-propionic and phenyl-acetic acid amongst the products of decomposition of certain albuminoid bodies.

Hydroparacumaric acid, $C_9H_{10}O_3$ $(HO.C_6H_4.CH_2.CH_2.CO.OH)$.

(Para-oxyphenyl-propionic acid).

This acid, besides being found amongst the products of the decomposition of proteids, is also found in urine.

Properties. When obtained by evaporation, the acid first of all presents the appearance of an oil, which subsequently crystallises. When recrystallised from water, it forms small, colourless, anhydrous, monoclinic crystals, which are readily soluble in water (especially hot), alcohol and ether. The solubility of this acid in water is greater than that of paroxyphenylacetic acid. Hydroparacumaric acid is sparingly soluble in benzol, but more soluble than paroxyphenylacetic acid. The melting-point of the acid is 125°—128° C. The zinc salt has the composition $(C_9H_9O_3)_2Zn + 2H_2O$ and crystallizes in pearly tables and leaflets, which are soluble in 130 parts of water at ordinary temperatures.

Reactions. With solutions of ferric chloride it gives a fleeting, but distinct, blue colouration. When boiled with Millon's reagent, the solution assumes a red colour and a red precipitate forms[1]. It does not reduce Fehling's solution.

Behaviour in the organism. When introduced into the organism hydroparacumaric acid is excreted in the urine in part as such, and in part as phenol.

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[1] Flügge shewed that phenol exhibits Millon's reaction (P. C. Flügge, 'Neue Reaction auf Carbolsäure,' *Zeitsch. f. anal. Chemie*, Vol. XI. (1872), p. 173. O. Nasse ('Ueber die aromatische Gruppe im Eiweissmolecül,' fully abstracted in Maly's *Jahresbericht*, Vol. IX. (1880), pp. 2—4, shewed that Millon's reaction is not confined to proteids and to tyrosin, but is a general reaction of all aromatic bodies in which a hydroxyl group is connected with the benzol ring.

Para-oxyphenyl-acetic acid, $C_8H_8O_3$ ($HO.C_6H_4.CH_2.CO.OH$).

This acid was obtained by E. and H. Salkowski[1] as a product of the putrefaction of wool and of albuminous bodies, and by Baumann from tyrosin by a process of putrefaction initiated by decomposing pancreas[2], besides being found by him in the urine, after injection of tyrosin, in phosphorus poisoning, &c.

Physical and chemical properties.

Para-oxyphenyl-acetic acid crystallises from water in the form of prismatic transparent prisms which are readily soluble in water, alcohol, and ether, but soluble with difficulty in benzol. It melts at 148° C. and when more strongly heated is partly decomposed and partly volatilises unchanged. The sulphates of copper, zinc and cadmium precipitate aqueous solutions of its ammoniacal salt.

Acetate of lead does not precipitate dilute solutions of the acid but when added to concentrated solutions occasions a crystalline precipitate, soluble in excess of the precipitant but which subsequently slowly separates out again. Under the influence of putrefaction, this acid decomposes, yielding, as products, parakresol and carbon dioxide.

Reactions.

With solution of iron chloride it gives a pale grey-violet, which soon changes to a dirty grey-green colouration.

It exhibits the red reaction when boiled with Millon's reagent.

Behaviour in the organism.

This acid, which, as has been said, is found in the urine after tyrosin has been given with the food, is excreted partly in the pure condition and partly in combination with sulphuric acid[3].

Phenyl-acetic acid, $C_6H_5.CH_2.COOH$ *and* *Phenyl-propionic acid,* $C_6H_5.CH_2.CH_2.COOH$.

These two acids were discovered in the products of the putrefactive decomposition of albuminous substances by the brothers Salkowski[3].

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[1] E. and H. Salkowski, *Ber. d. deutsch. chem. Ges.* Vol. XII. p. 650.

[2] Baumann, *Zeitsch. f. phys. Chem.* Vol. IV. (1880), p. 305.

[3] E. Salkowski, 'Zur Kenntniss der Pancreasverdauung (vorläufige Mittheilung),' *Zeitsch. f. physiol. Chemie*, Vol. II. (1878—79), pp. 420—424. In the 'Nachschrift' to this paper the Author announces that his brother had identified the acid of which it it treated as phenyl-acetic acid; see also E. and H. Salkowski, *Ber. d. d. chem. Gesell.* Vol. XII. (1879) pp. 107 and 653.

E. Salkowski, 'Zur Kenntniss der Eiweissfäulniss II. Die Skatolcarbonsäure,' *Zeitsch. f. phys. Chemie*, Vol. IX. (1885), pp. 8—22. The volatile aromatic acids are referred to and the scheme for their separation indicated (pp. 10—17),

E. Salkowski, 'Zur Kenntniss der Eiweissfäulniss III. Ueber die Bildung der nicht hydroxylirten aromatischen Säuren,' *Zeitsch. f. phys. Chemie*, Vol. IX. (1885), pp. 491—510.

The relative proportion of the acids present depends on the length of time during which the process of decomposition has been going on, phenyl-propionic acid being first formed. In the early stages, phenyl-acetic acid may be absent.

Besides being found amongst the products of the decomposition of albumin and gelatin by anaërobic bacteria (Nencki[1]), these acids have been detected in the contents of the *rumen* of the ox (Tappeiner[2]).

Method of separation[3]. The liquid products of putrefaction are distilled to one-sixth of their original volume and the residue is still further concentrated, then treated with alcohol and the alcoholic extract is filtered from insoluble matters. The alcoholic filtrate is evaporated to dryness, and the residue having been treated with water and rendered strongly acid, by means of sulphuric acid, is shaken up with ether. The ethereal solution is allowed to evaporate, and the residue is treated with solution of caustic soda until the reaction is alkaline. The liquid is heated, so as to dissolve the sodium soaps of the higher fatty acids and the hot, turbid, solution is precipitated by means of barium chloride. The mixture is filtered and the clear filtrate is evaporated to dryness, acidulated with hydrochloric acid, and extracted with ether. The ethereal solution is now evaporated to dryness, when an oily residue is left, consisting of volatile acids, oxy-acids, skatol-carbonic acid, succinic acid, &c. This residue is distilled in a current of steam, the distillate being collected in a solution of sodium hydrate. This alkaline solution is now concentrated, acidulated with hydrochloric acid and shaken up with ether. The residue left on evaporating the ether is distilled, and the fractions which distil at a temperature above 260° are collected apart; these fractions contain phenylpropionic and phenylacetic acids. In order to separate the two acids, the oily liquid is rubbed up with zinc oxide and water, and the magma is boiled, with considerable quantities of water, and filtered whilst yet hot. The insoluble matter contains the phenylpropionate of zinc, whilst the filtrate contains zinc phenylacetate, which separates out on cooling. By decomposing the respective zinc salts the pure acids are obtained.

Chemical characters. Phenylpropionic acid crystallises in long slender needles. Its melting-point is 47°—48° C. and it boils at about 280° C. Phenylacetic acid crystallises in broad leaflets. Its melting-point is 76·5° C. and it boils at 62° C. In accordance with their constitution, neither acid gives a red colouration or precipitate when heated with Millon's reagent.

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[1] Nencki, 'Untersuchungen über die Zersetzung des Eiweisses durch anaërobe Spaltpilze,' *Monatshefte f. Chem.* Vol. x. (1889), pp. 306 and 908.

[2] Tappeiner, *Zeitschrift f. Biol.* Vol. XXII. p. 236.

[3] Although the Author has carefully studied all the original papers bearing on this subject, in his description of the methods of separation of these bodies, he has availed himself of the succinct account given in Hoppe-Seyler's *Handbuch, &c.* 6te Auf. Berlin, 1893, p. 180.

Behaviour in the organism.

a. Phenyl-propionic acid. Phenyl-propionic acid appears in the first instance to be oxidised in the animal economy, and the benzoic acid, which is the result of this process, conjugating itself with glycerin, hippuric acid results, which is excreted in the urine. E. and H. Salkowski believe that the oxidation of the phenyl-propionic acid which is formed in the alimentary canal of carnivora as a product of the putrefactive decomposition of the proteids, is the source of the hippuric acid which they excrete in the urine[1].

b. Phenyl-acetic acid. When phenyl-acetic acid is introduced into the alimentary canal of dogs, it is absorbed and combines in the economy with glycocine, giving rise to a conjugate acid to which E. and H. Salkowski have assigned the name of phenaceturic acid.

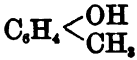
Phenaceturic acid $C_{10}H_{11}NO_3$ ($C_6H_5 . CH_2 . CO - NH . CH_2 . COOH$).

This acid, besides being produced when phenylacetic acid is artificially introduced into the organism of dogs, is a normal constituent of the urine of the horse and perhaps of that of man.

"It is sparingly soluble in water, though more soluble than hippuric acid; it is easily soluble in alcohol and acetic ether, but is sparingly soluble in ether.

"Its melting-point is 143° C. When boiled with hydrochloric acid, it is resolved into glycocine and phenyl-acetic acid[2]."

PHENOLS RESULTING FROM THE PUTREFACTIVE DECOMPOSITION OF TYROSINE.

Paralkresol.	*Phenol.*
 $C_6H_4<\begin{matrix}OH\\CH_3\end{matrix}$	C_6H_5OH
C_7H_8O	C_6H_6O.

Repeating the processes employed by Nencki in the preparation of indol from decomposing proteids, Baumann[4] arrived at the conclusion that appreciable quantities of a phenol are always produced during pancreatic putrefaction.

Weyl[5], at the suggestion and with the aid of Baumann, conducted experiments which led to the same result, but shewed that gelatin which, as Nencki had shewn, does not yield indol as a product of bacterial decomposition, also furnishes no phenol. Brieger[1] shewed that the excrements contain a phenol.

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[1] E. and H. Salkowski, 'Ueber das Verhalten der aus dem Eiweiss durch Fäulniss entstehenden aromatischen Säuren im Thierkörper,' *Zeitsch. f. phys. Chemie*, Vol. VII. (1882—83), p. 171.

[2] E. and H. Salkowski, *op. cit. Zeitsch. f. phys. Chem.* Vol. VII. (1882—83), p. 162.

[3] Hoppe-Seyler, *Handbuch, &c.* 6te Auf. Berlin, 1893, p. 182.

[4] Baumann, 'Zur Kenntniss der aromatischen Substanzen des Thierkörpers,' *Zeitsch. f. phys. Chemie*, Vol. I. (1877—8), pp. 60—69.

[5] Dr Th. Weyl, 'Fäulniss von Fibrin, Amyloid und Leim,' *Zeitsch. f. phys. Chem.*, Vol. I. (1877—78), p. 339.

Baumann's method of separating phenols from products of putrefaction.

Mixtures of albumin and pancreas are digested in the incubator at a temperature of 40° C., from 3 to 4 c.c. of a solution of ammonium carbonate being added to each litre. After 6 days' digestion, the liquid product is distilled, and the distillation continued so long as the distillate becomes distinctly turbid on the addition of bromine water. The strongly alkaline distillate is shaken up with one-half to three-fourths its volume of ether and the ethereal solution, having been collected apart by means of a separating funnel, is distilled. The residue is treated with caustic alkali and water and again distilled. The distillate consists of water, ammonia, indol and skatol. As soon as no more indol distils over, the residue in the retort is neutralised as carefully as possible with sulphuric acid and again distilled. The distillate, which contains the phenols, is abundantly precipitated on the addition of bromine water, and the precipitate very soon becomes crystalline, the crystals presenting the appearance of fine needles. If, instead of precipitating with bromine water, the distillate is shaken up with ether and the ethereal solution is evaporated, a residue is obtained which, when the pancreatic glands of several oxen have been employed, consists of some drops of an oily liquid; these possess an obvious smell of phenol and exert a caustic action when applied to the skin. When dissolved in water, the solution gives with ferric chloride a blue-violet colouration, and with ammonia and a particle of bleaching powder a beautiful blue reaction. Baumann recognised that the phenol thus obtained was not pure, and stated that, when precipitated with bromine, the tribromophenol obtained contained more than the theoretical quantity of bromine.

Baumann and Brieger's discovery of kresol in the products of putrefaction.

As it had been shewn that the urine of the horse contained the potassium salt of parakresol-sulphuric acid as well as that of phenol-sulphuric acid, it appeared likely that, in the process of putrefaction of proteids, parakresol might be produced. A research conducted by E. Baumann and L. Brieger proved the accuracy of the surmise[2]. Subsequently, working under Baumann's direction, Weyl succeeded in obtaining both parakresol and phenol by the putrefactive decomposition of tyrosine[3].

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[1] L. Brieger, 'Ueber die flüchtigen Bestandtheile der menschlichen Excremente' (aus d. Laborat. von Prof. Nencki), *Ber. d. d. chem. Gesell.*, Vol. x. (1877), p. 1027 *et seq.*

[2] E. Baumann und L. Brieger, 'Ueber die Entstehung von Kresolen bei der Fäulniss,' *Zeitsch. f. phys. Chem.*, Vol. III. (1879), p. 149.

[3] Th. Weyl, 'Spaltung von Tyrosin durch Fäulniss,' *Zeitsch. f. phys. Chem.*, Vol. III. (1879), p. 312.

Physical and chemical characters of phenol (carbolic acid), $C_6H_5 . OH$.

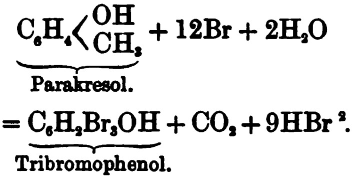
Phenol, when pure, crystallises in the form of rhombic needles; it melts at 40°—42° C., and boils at 180°—180·5°. It is soluble in 15 parts of water at 15°—17° C. It is easily soluble in alcohol, ether, and glycerin.

In aqueous solution, and in the absence of alcohol, phenol exhibits a violet reaction when treated with ferric chloride. It also exhibits Millon's reaction. With bromine water it yields a milky turbidity or a crystalline precipitate having the composition $C_6H_2Br_3OBr$. This body is soluble in solution of sodium hydrate, and the solution is precipitated on the addition of hydrochloric acid, which throws down tribromophenol, $C_6H_2Br_3OH$. This compound has a melting-point of 95° C. It contains 72·5 per cent. of Br. When boiled with Millon's reagent, an intense red colouration or a red precipitate is observed.

Physical and chemical characters of parakresol, $CH_3 . C_6H_4 . OH$.

Parakresol is much less soluble in water than phenol. It melts at 36° C. and boils at 198° C.

Aqueous solutions of parakresol are coloured blue by ferric chloride. With bromine it yields a crystalline precipitate which presents the form of small scales and not of needles[1]. When this precipitate is dissolved in alkalies and precipitated with hydrochloric acid, the body is found to be tribromophenol. It would appear, according to Baumann and Brieger, that under the influence of bromine, parakresol, like phenol, yields tribromo-phenol, CO_2 being evolved, as shewn in the following equation:

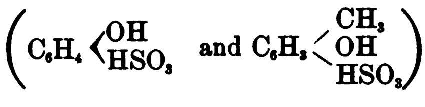


$$\underbrace{C_6H_4\langle\begin{smallmatrix}OH\\CH_3\end{smallmatrix}}_{\text{Parakresol.}} + 12Br + 2H_2O$$

$$= \underbrace{C_6H_2Br_3OH}_{\text{Tribromophenol.}} + CO_2 + 9HBr$$ [2].

Identification of phenol and parakresol.

In order to separate and identify phenol and parakresol these bodies must be converted into the respective sulphates



$$\left(C_6H_4\langle\begin{smallmatrix}OH\\HSO_3\end{smallmatrix} \quad \text{and } C_6H_3\langle\begin{smallmatrix}CH_3\\OH\\HSO_3\end{smallmatrix}\right)$$

by heating on the warm bath for an hour with an equal volume of strong sulphuric acid. The barium salts of these acids can be separated, the compound of parakresolsulphuric acid being almost insoluble, whilst that of paraphenolsulphuric acid is soluble[3].

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[1] Baumann und Brieger, *Ber. d. d. chem. Gesellsch.* 1879, p. 804: Weyl, *Zeitschrift für physiolog. Chemie*, Vol. III. (1879), p. 819.

[2] E. Baumann und L. Brieger, 'Ueber die Entstehung von Kresolen bei der Fäulniss,' *Zeitsch. f. phys. Chem.*, Vol. III. (1879), p. 149.

[3] For further details the reader is referred to Baumann and Brieger's paper in the

The kresols admit also of being identified by fusion with caustic potash[1]; orthokresol furnishes under these circumstances ortho-oxybenzoic (salicylic) acid, whilst pure kresol yields para-oxybenzoic acid.

Behaviour in the economy. The phenol and parakresol of the alimentary canal are converted in the economy into ethereal sulphates, viz. phenol is converted into phenol-sulphuric acid, and parakresol into kresol-sulphuric acid, which are excreted as potassium salts in the urine.

It is probable that these conjugate acids take their origin in the liver. The amount in which they are present in the urine appears to afford an indication of the intensity of the processes of decomposition due to bacterial action, which occur in the alimentary canal[2], [3].

NON-OCCURRENCE OF PTOMAÏNES[4] AS PRODUCTS OF NORMAL INTESTINAL DECOMPOSITION.

We have already alluded to the fact that the processes of the small intestine which are due to the bacterial decomposition of proteids differ materially from those of ordinary putrefaction, and no more striking proof of this assertion can be advanced than the fact that neither Brieger[5] nor Baumann and Udransky[6] were, *under normal circumstances*, able to find any of the so-called ptomaïnes in the intestinal contents, even when the intestines had not been opened for a day after death. That the intestinal contents contain, however, bacteria which are capable of producing ptomaïnes has been proved conclusively by the production of these bases in gelatin cultures of the intestinal bacteria.

We must therefore conclude that the peculiarity of the environment must be the cause which leads to a result so essential to the

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Zeitsch. f. phys. Chemie, Vol. III. p. 151, to a paper by Engelhardt and Latschinoff in *Jahresb. d. ges. Chem.* 1869, p. 447, and to Hoppe-Seyler's *Handbuch*, 6th ed. (1893), p. 158.

[1] Baumann und Brieger, *op. cit.* p. 150.

[2] Refer to pages 168 and 169.

[3] On the subject of the ethereal sulphates the reader may refer to the following papers: E. Baumann, Pflüger's *Archiv*, Vol. XIII. (1876), p. 297; E. Baumann, 'Ueber die Aetherschwefelsäuren der Phenole,' *Zeitsch. f. phys. Chem.*, Vol. 2, (1878—9), p. 335; Arthur Christiani and E. Baumann, 'Ueber den Ort der Bildung der Phenolschwefelsäure im Thierkörper,' *Zeitsch. f. phys. Chem.*, Vol. 2, (1878), p. 350; E. Baumann, 'Die aromatischen Verbindungen im Harn und die Darmfäulniss,' *Zeitsch. f. phys. Chem.*, Vol. x. (1886), p. 123.

[4] By the term *ptomaïnes* (from πτῶμα, a corpse, carcase) are designated nitrogenous bodies, for the most part having basic characters (of which many are intensely poisonous), which are produced as a result of the bacterial decomposition of dead bodies, and otherwise when proteids putrefy. These bodies will be treated of at length in another volume of this work, and are only incidentally referred to here.

[5] Brieger, *Deutsche med. Wochensch.* 1887, p. 469.

[6] Baumann und Udransky, 'Ueber das Vorkommen von Diaminen, sogenannten Ptomaïnen bei Cystinurie,' *Zeitsch. f. physiol. Chem.*, Vol. XIII. (1889), p. 586.

health and life of the animal as must be the non-production of ptomaïnes. It may be surmised that it is in this direction that the antiseptic influence of the bile acids makes itself felt, though it appears possible that the effect is due to the fact that ptomaïnes are essentially the products of anærobic bacterial processes, whilst it is probable that (notwithstanding the usual total absence of oxygen in the intestinal tube) a free diffusion of this gas is constantly going on between the blood of the mucous membrane and the intestinal contents which cover its surface.

Discovery of Cadaverin and Putrescin in the intestine in cystinuria, cholera and dysentery, pernicious anæmia.

Under very exceptional circumstances, however, bases which belong to the class of diamines have been found in the intestinal contents.

Thus Baumann and Udransky[1] have found that tetra-methylendiamin or putrescin, $NH_2-(CH_2)_4-NH_2$, and penta-methylendiamin or cadaverin, $NH_2-(CH_2)_5-NH_2$, regularly occur as products of intestinal decomposition in cases where cystin appears in the urine. The same diamines occur, according to Brieger[2], not only in cholera stools, but in cultures of the cholera spirillum. The two diamines to which we have referred are destitute of poisonous properties, and their presence merely indicates the occurrence of abnormal processes of decomposition; they appear to be accompanied, in the case of cholera cultures, by a very poisonous derivative of guanidin, the so-called methylguanidin, $CH_4N_3 . CH_3$, which is also a product of the putrefaction of muscle[3],[4] and by certain specific ptomaïnes, which possess the power of lowering the animal temperature.

SECT. 2. THE DECOMPOSITION OF THE CARBO-HYDRATES IN THE SMALL INTESTINE UNDER THE INFLUENCE OF BACTERIAL ACTION.

Although we have, in the last section, described at length the various products which can arise from the bacterial decomposition of the albuminous and albuminoid bodies, we have stated that in the small intestine such processes play a very insignificant part. With the bodies which belong to the group of the carbohydrates the matter is very different, for the small intestine is, throughout, the seat of processes of fermentation which decompose a part of the sugars which arise under the influence of the digestive enzymes, into simpler products. The chief of these are alcohol, lactic, acetic, and succinic acids, and, in certain cases, carbon dioxide and hydrogen.

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[1] Baumann und Udransky, 'Ueber das Vorkommen von Diaminen, sogenannten Ptomaïnen bei Cystinurie,' *Zeitsch. f. physiol. Chem.*, Vol. XIII. (1889), p. 562, and Vol. XV. 1891, p. 77.

[2] Brieger, Virchow's *Archiv*, Vol. CXV. p. 486.

[3] Brieger, 'Ueber Ptomaïne,' Dritter Theil, Berlin, 1886, p. 33.

[4] O. Bocklish, 'Ueber Ptomaïne aus Reinculturen von Vibrio proteus,' *Ber. d. deutsch. chem. Gesellsch.*, Vol. XX. p. 1441.

Designation	Distinguishing Morphological	Pathogenic? Special Remarks
I. **Bacterium Bischleri** ?Coli comm[illegible]	Short rods varying in length. On a 3 μ broad. Usually coupled. Do n movement. Formation of spores not B. coli com.	ogenic. When subcutane- njected killed guinea-pigs days products of fermentation it induces are the same se of B. coli com., save he latter generates dextro- lactic acid (sarcolactic
II. **Streptococcus liquefaciens ilei vel acidi lactici**	Small thin cocci, arranged in chai Readily stained by usual anilin colo they form small, round, yellow col narrow zone of liquefied gelatin. I after 24 hours, a turbidity occurs. Af streptococci	ogenic. Injections of n cultures caused death ours
III. **Bacterium ilei (Frey)**	Short rods with rounded ends, 2—3 often coupled. Mobility slight. F extremities. Easily coloured by me solution. Cultivated on gelatin, they and have a grey colour. Grow rapidl	
IV. **Bacillus liquefaciens ilei**	Fine, thin rods, 2·0—2·8 μ long and spores, grow rapidly and are mobile. On gelatin plates at ordinary tempera defined colonies which liquefy gela 'bouillon.' After 24 hours, liquid is a thin bacterial scum on the surface. putrefaction	
V. **Bacterium ovale ilei**	Almost circular and short (coccus on gelatin plates, round or oval colon Grow rapidly in 'bouillon,' without p	
VI. **Bacillus gracilis ilei**	Fine, thin rods, about 5 times a mobile, usually coupled. Formation On gelatin plates, whitish-yellow, ro borders. Grow well in 'bouillon' at 3	
VII. **Bacterium lactis aërogenes (Escherich)?**	Short rods with rounded ends. S often aggregated in masses. On gelat which, on the surface, appear white an layers, yellowish-white, round, poin 'bouillon.'	ogenic. Kills guinea-pigs days

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[1] Macfadyen, Nencki u. Sieber, 'Untersu 0.

To face p. 437.

As we shall have occasion to point out in the next chapter, when considering the processes which have their seat in the small intestine as a whole, so great is the quantity of organic acids formed by the organised ferments of the small intestine that in spite of the sodium carbonate which the mucous membrane is, doubtless, perpetually secreting during digestion, the contents of the small intestine from pylorus to cæcum are, in opposition to the hackneyed teaching of the schools, always acid.

Many researches have, of recent years, been conducted on the micro-organisms of the small intestine and on the fermentations to which they give rise, and of these the most important is that conducted by Nencki, Macfadyen and Sieber, the mean results of which have been summarised by the Author in the accompanying table, to which the reader is referred. It will be seen that these investigators were able to identify seven individual micro-organisms which give rise to fermentations in the small intestines. Of these only two excited any action on the albuminous substances (*Streptococcus liquefaciens ilei* and *Bacillus liquefaciens ilei*). With the exception of the last-named, all the six others exert a powerful action on dextrose. Five of the seven decompose dextrose with the production of alcohol, often in large quantities; six of the seven produce lactic acid. Amongst them, the most active is the *Streptococcus liquefaciens ilei*, which when acting on a solution of dextrose, converts nearly the whole of the sugar into inactive lactic acid (ordinary lactic acid of fermentation, optically inactive ethylidene-lactic acid[1]).

The conversion of dextrose into lactic acid under the influence of ferments is a process of simple decomposition which is identical with that which occurs when it is heated with caustic alkalies, and it may be represented by the following equation:

$$C_6H_{12}O_6 = 2\,(CH_3 - CH\,.\,OH - COOH).$$

The conversion of dextrose into alcohol, if we leave out of consideration the very numerous by-products which are formed in the process and confine our attention merely to the two principal products formed (alcohol and CO_2), may be represented by the following equation:

$$C_6H_{12}O_6 = 2\,(C_2H_5\,.\,OH) + 2CO_2.$$

Although Nencki's researches do not give any facts bearing on the question, it is probable enough that the butyric acid fermentation occurs in the intestine. Under the influence of certain of the ferments, particularly of the *Bacterium ilei* (Frey), the products of fermentation separated were succinic acid, sarcolactic acid and alcohol, large quantities of a mixture of CO_2 and H being evolved.

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[1] See Vol. I. (1st edit.), p. 362.

The formation of butyric acid from dextrose is represented by the equation

$$C_6H_{12}O_6 = C_4H_8O_2 + 2CO_2 + 2H_2.$$

But butyric acid may also be developed by the action of ferments on lactic acid, thus:

$$2(C_3H_6O_3) = C_4H_8O_2 + 2CO_2 + 2H_2.$$

SECT. 3. THE DECOMPOSITION OF THE FATS IN THE SMALL INTESTINE, UNDER THE INFLUENCE OF BACTERIAL ACTION.

Whilst there can be no doubt that under the influence of putrefactive organisms fats may become rancid, *i.e.* be decomposed into fatty acids and glycerin, there does not appear evidence to support the view that such a process occurs, or at least attains a perceptible figure, in the small intestine[1]. Lecithin is said to be decomposed into glycerin-phosphoric acid and choline, the latter substance being further decomposed into carbonic acid, marsh gas and ammonia[2].

SECT. 4. THE GASES OF THE SMALL INTESTINE.

The small intestines are always more or less distended with gases, which are composed of carbon dioxide, hydrogen, and nitrogen, marsh gas being absent. The following are the results of the gases of the small intestine of the dog made by Planer[3].

	I. On a meat diet	II. On a bread diet	III. On a leguminous diet
CO_2 in 100 volumes	40·1	38·8	47·3
H	13·9	6·3	48·7
O	0·5	0·7	0
N	45·5	54·2	4·0

K. B. Hofmann also analysed the gases of the small intestine of the dog and rabbit, and found them to be composed of a mixture of CO_2, H and N, neither O nor CH_4 being present[4].

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[1] Macfadyen, Nencki u. Sieber, *op. cit.*, p. 347.

[2] Hasebroek, *Zeitsch. f. physiol. Chemie*, Vol. XII. (1888), p. 148.

[3] Planer, *Sitzungsber. d. Wien. Akad. d. Wiss.*, Vol. XLII. (quoted at second hand).

[4] K. B. Hofmann, 'Ueber die Zusammensetzung der Darmglase,' *Wiener med. Wochenschrift*, 1872, No. 24; Maly's *Jahresbericht*, Vol. II. (1874), p. 226.

CHAPTER XI.

A BRIEF SURVEY OF THE PROCESSES OCCURRING IN THE SMALL INTESTINE, IN RELATION ONE TO THE OTHER. THE DESTRUCTION OF THE DIGESTIVE ENZYMES.

HAVING considered, in detail, the action of the various secretions which are poured into the alimentary canal, on the various constituents which the chyme contains on leaving the stomach, we must now attempt very briefly to combine the various facts and to supplement them by reference to some, the consideration of which it appeared desirable to postpone.

Newly-discovered facts tending to prove that none of the water ingested is absorbed by the stomach, but passes into the intestine.

Although the subject of absorption will not be minutely discussed in this place, it is necessary to refer to researches published since the first chapters of the present volume were printed, which, in opposition to the statement made at page 154, prove that the stomach absorbs no water, so that the whole of the water of the food passes during the digestive process into the intestine. The matter has so important a bearing on the whole conception of intestinal digestion as to warrant the attention of the reader being drawn to it in the present place.

Tappeiner's researches.

Tappeiner[1], as a result of experiments on dogs and cats in which he had ligatured the pylorus before injecting into the stomach solutions of various alimentary and poisonous substances, came to the conclusion that the stomach absorbs only very small quantities of bodies which are introduced into it in aqueous solution, whilst, on the other hand, it appears readily to absorb dilute alcohol and substances dissolved in alcohol. Thus the quantities of peptone, glucose and sodium sulphate absorbed from aqueous solutions were so small as to fall within the limits of experimental error When a dose of an aqueous solution of alcohol, sufficient to induce deep sleep in the course of 10 minutes (when administered to a dog with unligatured pylorus), was administered to one in which it had been ligatured, scarcely any physiological action ensued.

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[1] H. Tappeiner, 'Ueber Resorption im Magen,' *Zeitsch. f. Biol.*, Vol. XVI. (1881), pp. 497—507.

Again, when sulphate of strychnia was injected into the stomach of cats with ligatured pylorus, death from strychnia poisoning ensued in a period varying between one and a half and three hours afterwards, whilst with unligatured pylorus death occurred from the same dose in 8 minutes. If, however, strychnine were dissolved in dilute alcohol (0·04 grm. of strychnine dissolved in 5 c.c. of 90 p.c. alcohol and 15 c.c. of water) and were injected into the stomach of a cat with ligatured pylorus, death occurred in ten minutes, *i.e.* in the same time as when the same dose was administered to an animal in the normal condition. The conclusion to which Tappeiner arrived was that the absorptive power of the stomach is greatly inferior to that of the intestine in so far as substances dissolved in water are concerned, but that it possesses the power of absorbing dilute alcohol as well as substances dissolved in it.

The investigation of Anrep and Meade Smith.

Anrep[1] in Ludwig's laboratory, repeated Tappeiner's experiments on dogs with gastric fistulæ but came to the conclusion that both sugar and proteids are absorbed by the stomach in considerable quantities. Meade Smith[2] introduced solutions of sugar as well as of proteids into the stomach of frogs in which he had some time before ligatured the pylorus. The quantity of sugar absorbed varied with the concentration of the solution. Meade Smith, however, found that the quantity of liquid present in the stomach at the conclusion of the experiment was greater than that which he had introduced. So far as one can draw conclusions from experiments in which the normal mechanism is so materially disturbed, the absorption of water from the stomach appeared to be very doubtful[3].

The experiments of J. S. Edkins.

The experiments which have been passed under review, with the exception of those of Meade Smith, concerned directly only the absorption of substances dissolved in water or in alcohol and gave no direct answer to the question does the stomach absorb the water introduced into it, or does the latter entirely pass into the duodenum? In the year 1892 J. S. Edkins published the results of a research which led him to the conclusion that, whether the stomach had been digesting or not, "the absorption (of water) was practically nothing[4]."

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[1] B. v. Anrep, 'Die Aufsaugung im Magen des Hundes' (aus d. phys. Anstalt zu Leipzig), Du Bois Reymond's *Archiv*, *Phys. Abth.*, 1881, p. 504 *et seq.*

[2] R. Meade Smith, 'Die Resorption des Zuckers und des Eiweisses im Magen' (aus d. phys. Anstalt zu Leipzig), Du Bois Reymond's *Archiv*, *Phys. Abth.*, 1884, pp. 481—496.

[3] See also M. Segall, 'Versuche über die Resorption des Zuckers im Magen,' *Inaug. Diss.* München, 1888. Abstracted in *Centralbl. f. d. med. Wissensch.*, 1889, p. 610, and Maly's *Jahresbericht*, 1889, p. 281.

[4] J. T. Edkins, M.A., M.B., 'The Absorption of Water in the Alimentary Canal'

Although, as we shall shew in the sequel, the conclusion to which Edkins arrived was correct, the method of experiment adopted was of such a nature as to inspire great and reasonable doubts as to whether it was applicable to animals in the physiological condition. Having injected into a cat 25 minims of a solution of morphia and atropine containing half a grain (0·032 grms.) of hydrochlorate of morphia and one-thirteenth of a grain of sulphate of atropia (0·0084 grm.), and having placed the animal under chloroform, Edkins ligatured tightly the cardiac end of the stomach and then tied a cannula, communicating with a reservoir, into the pylorus. Employing a normal saline solution, he found, as stated above, that the absorption of water was virtually *nil*. To these experiments, quite apart from the violence of the treatment which the stomach necessarily suffered, the strongest objection which can be urged is afforded by the fact that they were performed on animals to which, in addition to morphia, a very large dose of atropia had been administered. The disturbing influence of this drug is such that, in the opinion of the Author, the result obtained would in no respect warrant our admitting the accuracy of Edkins's conclusion, were it not for the fact that a beautiful research of v. Mering's has now established it beyond the possibility of doubt.

v. Mering's researches[1].

In cases of marked dilatation of the stomach depending upon pyloric stenosis, the patient usually suffers from thirst, passes little urine, is constipated, and has a dry skin, phenomena which have been explained on the hypothesis that fluid is absorbed with difficulty by a dilated stomach and, besides, that little can pass out of the stomach into the intestine when the pylorus is contracted. The stomach of such patients, unless the contents are artificially evacuated, usually contains large quantities of liquid. If in such a case the stomach be emptied—for example in the evening—and food, consisting, *e.g.* of a thick soup, be introduced into it, the following morning the stomach is still found to contain liquid: often indeed more, and of a lower specific gravity, than that which had been introduced the previous evening[2].

Reasoning on these clinical facts, v. Mering, who appears to have been entirely ignorant of Edkins's research, engaged in the experiments now to be referred to. In the first instance, in the case of large dogs, he cut across the duodenum, from five to ten centimetres below the pylorus, and sewing each end into the skin he obtained two fistulous apertures, the upper leading to the pylorus and the lower into the duodenum. When the animal had recovered from the effects of the

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(from the Physiological Laboratory of the Owens College), *Journal of Physiology*, Vol. XIII. (1892), pp. 445—459. See pp. 454—459.

[1] Professor Dr J. v. Mering (Halle), 'Ueber die Function des Magens' (Unter Mitwirkung von Dr Aldehaff und Dr Happel). Separatabdruck aus den 'Verhandlungen des XII. Congress für innere Medicin zu Wiesbaden 1893,' Wiesbaden. Verlag von J. F. Bergmann, 1893.

[2] J. v. Mering, *op. cit.* p. 4.

narcosis and the operation, water was given to it. As it drank, water flowed out of the stomach, but always rhythmically and in distinct gushes. On introducing the finger, it was easy to feel that the pylorus opened and closed at short intervals. Each minute, the pylorus opened from two to six times, and each time expelled several cubic centimetres (2—15) of water. The outflow occurred under considerable pressure and lasted some seconds: then came a long pause which was followed by another gush of water, and so on. By this experiment the remarkable fact was established that (within a few c.c., more or less) the whole quantity of water introduced into the empty stomach flowed out of it. In accordance with the above statement, it was found that an animal upon which such an operation had been performed could not quench its thirst by drinking; on the contrary, the more it drank the more thirsty it appeared to be. This fact is explicable by supposing that, in consequence of a small amount of secretion occurring, the stomach actually lost water.

By injecting 300 to 500 c.c. of warm milk three times daily into the duodenal fistula, the animal could be fed and, for a few days, appeared to enjoy perfectly good health. After a period, varying between three and eight days, there supervened, however, a remarkable association of symptoms:—twitchings of the extremities and of the facial muscles, the limbs became rigid, the teeth chattered, the pupils often became dilated, and paralytic or paretic symptoms, with constantly heightened tendon reflexes, made their appearance; these were soon followed by somnolence, deep respirations and, ultimately, by death. This association of symptoms corresponds, v. Mering remarks, with those of the condition which Kussmaul described a quarter of a century ago under the name of gastric tetany ('Magentetanie') and which has since been frequently observed and described by Gerhardt, F. Müller, and others.

As all the animals which had been subjected to the operative procedure above referred to, died in the way described, v. Mering next established a duodenal fistula, in close proximity to the pylorus. Having made a suitable incision into the abdominal wall and drawn out the stomach and duodenum, he fixed the duodenum at the spot selected (5—8 cm. below the pylorus) to the wound in the abdominal wall. With this object, 6—8 sutures were introduced which passed through the serous and muscular coats of the intestine on the one hand, and through the parietal layer of the peritoneum on the other. After three or four days, the wall of the intestine enclosed between the sutures was incised down to the mucous membrane, fresh stitches being introduced through the serous and muscular coats. One or two days afterwards, the mucous membrane was incised and the communication with the interior of the gut established. During each experiment, the passage downwards into the small intestine was closed by means of a small caoutchouc bag distended with water. In this second set of researches the results obtained by the first were confirmed and extended. In one case, 500 c.c. of water being administered to a large dog, 490 c.c. were expelled in the succeeding 20 minutes; in another, 25 minutes after the administration of 500 c.c., 495 c.c. were expelled.

Experiments further proved that *a state of repletion of the small intestine reflexly slows the evacuation of the stomach*, and that psychical excitement also inhibits its evacuation.

By further observations, v. Mering shewed that when water holding CO_2 in solution is introduced into the stomach the gas is abundantly absorbed. Alcohol he found to be abundantly absorbed. Grape-sugar, milch-sugar, cane-sugar, and maltose, when in solution in water, are all absorbed by the stomach in considerable quantities, and in larger quantities when in alcoholic solution. Dextrin and peptone are absorbed by the stomach, but in smaller quantities than the sugars. The quantity of the bodies absorbed increases with the concentration of the solutions. *Pari passu* with the process of absorption of the bodies above named, there occurs an excretion of water by the stomach which is active in proportion to the amount of substance absorbed.

Recapitulation of the Chemical Processes occurring in the Small Intestine.

We have seen that, at rhythmically recurring intervals, during the process of gastric digestion, the fluid part of the contents of the stomach is, little by little, expelled into the duodenum. Ultimately (see p. 159) the more or less diffluent '*chyme*,' containing both soluble constituents which had escaped absorption in the stomach and insoluble constituents not yet acted upon, is forced through the pylorus. Coming into contact with the bile and the pancreatic juice, peptic digestion comes to an end and digestion by trypsin commences. The proteids which had escaped the action of the gastric juice now succumb to the action of trypsin. The digestion of the starches, which had been arrested in the stomach, recommences under the influence of the diastatic ferment of the pancreas, and the maltose thus formed has to be resolved into simpler saccharine molecules by a ferment-product of the intestinal wall; these molecules must yet, in part, still further be resolved under the influence of lactic acid producing micro-organisms which they encounter. The fats, under the influence of the bile aided by the pancreatic juice, are rapidly emulsified. As these various operations go on, the contents of the intestine undergo a great and rapid diminution, owing to the absorption of water, holding the diffusible products of digestion in solution, and to the passage of the minutely-subdivided fats through the intestinal walls.

The destruction of pepsin. As the acid chyme penetrates into the duodenum it undergoes at once a change of reaction due to admixture with the pancreatic juice, the bile and the intestinal juice. Its reaction becomes alkaline or at least ceases to be distinctly acid.

This neutralisation of free acids would of itself bring peptic digestion to a standstill, even in the absence of other factors.

We have already pointed out (see page 352) that one principal result of the admixture of the acid chyme with the bile is the precipitation of the pepsin of the gastric juice, the digestive activity of which is *ipso facto* abolished (Pappenheim, Brücke). It appears that, once rendered inactive by admixture with bile, pepsin cannot regain its activity. The fact that digestion may in the stomach go on in the presence of some bile is in no sense in contradiction with the statement as to what must take place in the intestine. In the stomach a continual secretion of pepsin can occur, to replace that which has been rendered inactive, *e.g.* by the bile which may have penetrated into the stomach.

But, in addition to its more or less complete precipitation by the bile, the pepsin which has entered the duodenum finds itself subjected to the action of the Na_2CO_3 of the pancreatic juice. As Kühne[1] first pointed out, and as Langley[2] subsequently confirmed, pepsin is destroyed by digestion with weak alkaline solutions. The latter observer shewed that solutions of sodium carbonate, at the temperature of the mammalian body, exerted a powerfully destructive influence on pepsin.

It is impossible to estimate exactly the relative part played by the bile on the one hand, and the sodium carbonate on the other, in putting the pepsin *hors de combat*, but we may surmise that it is upon the bile that the burden of the work chiefly falls. Whilst the action which it exerts on the mixture of pepsin, albumoses and free acid is a well-nigh instantaneous one, that of the sodium carbonate is a gradual one, time being one of the important elements influencing the result.

The paramount importance of this destruction of pepsin is apparent when we reflect that pepsin, in the presence of free acids, exerts a rapid destructive influence on trypsin.

Corvisart[3] had stated that pepsin and trypsin exert a mutually destructive influence on one another. Kühne, whilst emphasising strongly the power of pepsin in acid solution to destroy trypsin, spoke of the indestructibility of pepsin by pancreatic digestion[4]. Langley[5], however,

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[1] W. Kühne, 'Ueber das Verhalten verschiedener organisirter und sog. ungeformter Fermente.' Separat-Abdruck aus den *Verhandlungen des Heidelberg. naturhist. med. Vereins.* Sitzung am 4. Feb. 1876.

[2] J. N. Langley, 'On the Destruction of Ferments in the Alimentary Canal,' *Journ. of Physiology*, Vol. III. (1880—82), pp. 246—268.

[3] L. Corvisart, 'Mais c'est une chose remarquable que si les deux ferments digestifs se rencontrent à l'état pur, les deux digestions cessent de s'exercer aussi librement; loin que le produit digéré soit doublé par cette réunion, au contraire il peut se réduire à rien, car dans cette circonstance non physiologique, la pepsine et la pancréatine s'entre-détruisent.' *Sur une fonction peu connue du Pancréas &c.* Paris, Victor Masson, 1857—58. See p. 116.

[4] W. Kühne, 'Die Unzerstörbarkeit des Pepsins bei der pankreatischen Verdauung,' *op. cit.*, p. 5.

[5] J. N. Langley, *op. cit.*

found that pepsin was more rapidly destroyed by a solution of sodium carbonate containing trypsin than in one free from it, and discovered that pepsinogen also appeared to be destroyed in the same manner.

Were it not for the arrest of peptic proteolysis, the intestinal digestion by the pancreatic trypsin would be impossible. With the admixture, then, of the acid chyme with the bile and with the pancreatic juice, the first act in the great process of digestion may be said to come to an end.

We have devoted so much space to a consideration of the action exerted both by the bile and the pancreatic juice on the various groups of food constituents that it is only necessary in this place to emphasize certain facts as of special importance, and to supplement them by referring to others which have inadvertently been omitted.

Digestion by trypsin as it proceeds in the intestine resembles an aseptic pancreatic digestion as conducted *in vitro*, with the aid of such agents as thymol and salicylic acid, rather than one occurring in the presence of putrefactive bacteria. We have seen that the contents of the small intestine are destitute of putrefactive odour, that they are normally destitute of the micro-organisms which lead to the production of indol, skatol, skatol-carbonic acid and phenols, &c. The interesting question remains, however, how far, in the physiological condition, does the decomposition of the albuminous molecule proceed under the influence of trypsin? We have seen that, *in vitro*, the albuminous bodies rapidly split up into albumoses and peptones, and that the latter even in the absence of all organisms, undergo, in part, ready decomposition, yielding as principal products amido-acids, lysine and lysatinine and tryptophan. It appeared probable enough that in the conditions existing in the alimentary canal the decomposition might not proceed so far—that hemipeptones and antipeptones once formed, might, either as such or as regenerated albumins, pass through the intestinal wall, without undergoing further degradation. The experiments of Sheridan Lea have shewn that even in the normal living alimentary canal of the dog, a fraction of the peptones, and not altogether an insignificant one, undergoes the decomposition which leads to the setting free of the amido-acids[1]. On the other hand, Macfadyen, Nencki and Sieber failed to find leucine and tyrosine in the contents of the ileum and explained the fact on the hypothesis that the lactic acid, which is generated through the instrumentality of the micro-organisms, modifies the activity of trypsin-digestion sufficiently to prevent so profound a decomposition of the albuminous molecule as is implied by the appearance of the amido-acids.

In so far as the action of the diastatic enzyme of the pancreas is concerned, we may now, with confidence, assert that it results in the formation of maltose, and that it is not capable of breaking down this complex sugar into the simpler monosaccharides. The salivary and pancreatic diastatic enzymes perform, in respect to the starches and dextrins, a function of which the complement is performed chiefly by the maltose-converting enzyme of the intestinal wall and, in a less degree, by the lactic acid producing organisms which find a suitable habitat in the small intestine.

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[1] Sheridan Lea, 'A Comparative Study of Artificial and Natural Digestion,' *Journ. of Physiology*, Vol. XI. (1890), p. 227 *et seq.*

In reference to the digestion of fats, the pancreas seems to play a part accessory, though perhaps subordinate, to that which the bile discharges. By the fractional decomposition of the neutral fats which it exerts, in virtue of its fat-splitting ferment, it establishes the conditions which are favourable to the formation of a true emulsion by the bile.

Amongst the enzymes of the intestinal canal, curdling ferments must not be forgotten. The mucous membrane of the small intestine, throughout its course, appears to form such a ferment, which is, in all probability, also present in the pancreatic juice, although so far as the Author is aware, no direct experimental evidence of the fact exists. Roberts, Harris and Gow, and others, have drawn attention to the fact that all extracts of the pancreas curdle milk, and seem to assume that we may logically conclude that the pancreatic juice exerts a similar action. But that the inference is not absolutely justified results from the fact that, although the mucous membrane of the stomach of all adult animals yields, on suitable treatment, extracts which curdle milk, it is only in very exceptional cases (as in that of man) that the gastric juice of adult animals possesses that property.

The function of the lactic acid fermentation in the intestine.

Attention has been drawn to the error in which nearly all systematic writers have fallen in their account of the reaction of the contents of the small intestine. If we except the duodenum at the very time when pancreatic juice is being most rapidly secreted, there can be no doubt that the intestinal *contents* are acid. The acid reaction is, as we have shewn, the result of the production of lactic acid, at the expense of a part of the sugar formed in the alimentary canal, and is effected through the agency of various organisms (see p. 437). There can be no doubt that a part of the lactic acid formed must at once enter into combination with the sodium carbonate which is so prominent a constituent of the intestinal juice. The excess must play a leading part, both in virtue of its own influence and by setting free the bile acids, in preventing putrefactive processes occurring in the small intestine, to the danger of the health, and to the risk of the life, of the creature.

The destruction of trypsin and other enzymes in the small intestine.

So long as it was believed that the contents of the small intestine presented, normally, an alkaline, and the contents of the large intestine an acid, reaction, it was believed that the pancreatic enzymes, which are all destroyed by digestion with dilute acids, must be rendered inactive when passing from the ileum into the colon. Seeing that the contents of the small intestine possess normally an acid reaction, which (presumedly) increases from above downwards, it may be assumed that the pancreatic enzymes are destroyed before the ilio-cæcal valve is reached. The observations which have been made on the fluid obtained from fistulæ of the ileum in the vicinity of the cæcum leave no room for doubt on this question.

Paths of absorption. It is the intention of the Author to defer the detailed consideration of all the questions connected with the absorption of the products of digestion, and he will, therefore, confine himself in this place to the most rudimentary facts.

It is in the small intestine that absorption of the dissolved organic solids of the food chiefly occurs. The large surface of the mucous membrane of this part of the alimentary canal, with its innumerable villi, offers an absorbing surface of large extent, pervaded by meshworks of capillaries and by the commencement of the lymphatics, the so-called 'lacteals.' In considering the extent of this surface the 'valvulæ conniventes' must not be forgotten. These so-called valves are crescentic folds of the mucous membrane, which is doubtless arranged in this manner so as to afford, in a given area, a larger extent of absorbing surface than would otherwise be possible.

The absorption of materials from the alimentary canal takes place by their passage into the blood capillaries and, in part, by their passage into the lymphatics of the mucous membrane (lacteals). Water, inorganic salts, sugars, fatty acids and their salts, albumoses, peptones and the products of their decomposition, and emulsionized fats are the principal substances present in the intestine. The paths of absorption followed by these various substances are not the same. Whilst the fat makes its way into the lacteals, the other constituents, *more or less changed in their passage*, find their way into the capillaries and thence directly into the blood. Even in this strictly rudimentary reference to intestinal absorption, it is necessary to mention that although albumoses and peptones abound in the contents of the intestine, these bodies can neither be detected in the lymph nor in the blood. Either a reconversion of albumoses and peptones occurs within the alimentary canal itself (as Kronecker and his school have contended), or the reconversion takes place as the albumoses and peptones are brought in contact with the epithelial layer covering the villi, or are subjected to the action of the leucocytes of the adenoid tissue. These various views will be examined and discussed in the next volume.

CHAPTER XII.

THE LARGE INTESTINE AND THE PROCESSES WHICH HAVE THEIR SEAT IN IT. THE FÆCES IN HEALTH AND DISEASE. THE INTESTINAL GASES. INTESTINAL CONCRETIONS.

SECT. 1. PRELIMINARY OBSERVATIONS ON THE ARRANGEMENT AND STRUCTURE OF THE LARGE INTESTINE.

As we trace the small intestine from its commencement at the pyloric end of the stomach we find that this long tube, which in man possesses an average length of 20 feet, gradually diminishes in calibre as we pass from duodenum to jejunum and from jejunum to ileum. At the lower end of the latter the small opens suddenly into the much wider, large intestine or '*colon:*' though not at the very commencement of this, which is a cul-de-sac (the *caput cæcum coli* or *cæcum*), but at a point a little removed from this. The margins of the aperture by which the small intestine opens into the large, project into the latter in such a manner that, while they readily permit the passage of matters from small into large intestine, any backward movement of the contents of the large intestine would have the effect of compressing the lips of the opening and closing it; this arrangement constitutes the *ilio-cæcal* or *ilio-colic* valve. Connected with the *caput cæcum coli* is (in man and monkeys) a small diverticulum, like a narrow glove-finger, called the *vermiform appendix.* The first and greater part of the large intestine is known as the *colon,* the last as the *rectum.* The colon is subdivided into *ascending* colon, *transverse* colon, and *descending* colon, the bend made by the transverse in passing into the descending colon, receiving the name of the *sigmoid flexure.* The total length is from five to six feet.

The arrangements of the coats of the large intestine are similar to those of the small, though modifications in each of the constituent coats are obvious.

The mucous membrane is characterised by the absence of villi. It presents innumerable glands built on the type of the glands of Lieberkühn of the small intestine, but very much larger and especially much longer than these and possessing a wider *lumen.* Their epithelium cells present, in a very characteristic manner, large numbers of 'goblet-cells.'

The muscular coat exhibits a peculiarity in the arrangement of its bundles of fibres, especially of those of its longitudinal coat, which are 'gathered up into three thickened bands or bundles, being very thin elsewhere. These bands, moreover, are shorter than what may be called the natural length of the intestine, so that the tube instead of being, as in the small intestine, of fairly uniform bore, is puckered up into sacculi more or less divided by the three bands into groups of three. This sacculated arrangement answers much the same purpose as the arrangement of valvulæ conniventes in the small intestine. The circular muscular layer is thicker in the middle or bellies of the sacculi than at the puckers, where it is very thin...As the sigmoid flexure passes into the rectum, the three bands of the longitudinal muscular layer spread out and become once more a uniform layer; and with this change the sacculation disappears. This longitudinal coat is continued to the anus, where it ends abruptly. The circular coat at its termination at the anus is developed into a distinct ring, the internal sphincter...Down to the margin of the anus the mucous membrane retains the characters of the large intestine, glands being still present; it then abruptly puts on the epiblastic characters of the epidermis[1].'

SECT. 2. THE CHARACTERS OF THE INTESTINAL CONTENTS AS THEY PASS FROM THE ILEUM INTO THE LARGE INTESTINE.

As the contents of the ileum pass into the colon they are more or less diffluent, possess a yellowish colour, and are almost or quite devoid of odour. From the admirably studied, and almost unique case of Macfadyen, Nencki and Sieber[2], in which a fistula of the ileum existed at its very junction with the colon, it would appear that the contents of the small intestine are continually passing into the large, though the flow is less during the night, apparently in consequence of the abstention from food. Macfadyen, Nencki and Sieber determined how long ingested bodies occupied in reaching the cæcum, and they found the time to vary within wide limits. When their patient ate green peas, the first of these appeared at the fistulous aperture, in one case 2¼, and in another 5¼ hours afterwards. In the first case, the last of the green peas were discharged 14 hours, in the second case 23 hours, after they had been swallowed. The rate with which the intestinal contents travel towards, and the time occupied in reaching, the ilio-cæcal valve depend upon the consistence of the intestinal contents and upon the intensity of the intestinal movements.

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[1] M. Foster, *A Text-Book of Physiology*, 5th ed. Part II. comprising Book II. pp. 450 and 451. Macmillan, 1889.

[2] Macfadyen, Nencki and Sieber, 'Ueber die chemischen Vorgänge im menschlichen Dünndarm,' *Archiv f. exp. Path. u. Pharm.*, Vol. XXVIII. (1891), p. 311 *et seq.*

The morphological constituents of the intestinal contents at the entrance into the colon.

When fed upon a diet composed of bread, meat, Kemmerich's 'peptone,' sugar, milk, beef-tea and eggs, the intestinal contents exhibited (under the microscope) many bile-stained, striped, muscular fibres: masses of "detritus": pigment granules: amorphous flakes of albumin, mucin and bile acids: vegetable fibres: and numerous bacteria. When the patient was fed upon a diet composed mainly of pease-porridge and containing therefore a large quantity of starch, on microscopic examination, the granules of the latter body preponderated; iodine stained them, however, of a red colour.

The chemical characters of the intestinal contents when passing into the colon.

The reaction of the intestinal contents, as they leave the ileum to enter the cæcum, is acid, the acidity being on an average equal to that of a solution of acetic acid containing 1 part in 1000, and depending upon the presence of organic acids, amongst which preponderates the inactive lactic acid of fermentation. The fluid part of the intestinal contents, filtered from solid matters, contains the following constituents:—Albumin coagulable by heat, mucin, peptones, (and albumoses?), the products of transformation of starch, inactive lactic acid of fermentation, as well as the optically active paralactic acid, small quantities of volatile fatty acids (principally acetic acid), bile acids and bilirubin (*not hydrobilirubin!*). When exposed to the air, the intestinal contents assume a green colour in consequence of the conversion of bilirubin into biliverdin. When the activity of the filtrate is sensibly greater than corresponds to 1 per 1000 of acetic acid—say 1·5 to 2·0 per 1000—the addition of acetic acid occasions no precipitate: at most a faint turbidity. If the quantity of acid be small, acetic acid throws down mucin in a flocculent condition. In consequence of its containing free acid, the albumin in the filtrate of the intestinal contents coagulates on mere boiling. When, however, the acidity is great, the filtrate must be partly neutralised before the albumin can be thrown down on boiling.

Quantities of available alimentary principles in intestinal contents passing into colon.

Macfadyen, Nencki and Sieber found that when their patient was fed upon a mixed diet and the intestinal contents were thin, they contained about 5 per cent. of total solids; when the contents were more concentrated they contained, on an average, 10 per cent. of total solids. Some idea of the total quantity of the contents passing from the ileum in 24 hours may be formed by the results of two observations. The total quantity being 550 grms., the residue weighed 4·9 per cent.; the total solids leaving the ileum being therefore 26·95 grms. On another occasion, 232 grms. were collected, containing 11.23 per cent. of residue, the total solid matters amounting, therefore, to 26·05 grms.

When we now inquire in what proportions the various groups of alimentary constituents are present in the matters which pass into the colon, we find that coagulable albumin amounts to less than

1 per cent. The sugar exhibits much greater variations than the albumin, varying between 0·3 and 4·75 per cent.

Macfadyen, Nencki and Sieber estimate that from 30—42 per cent. of the solid matters of the intestinal contents entering the colon are composed of albuminous matters (including native albumins, albumoses and peptones, insoluble albuminous and albuminoid matters), about 8·5 per cent. of fats, about 45 per cent. of carbohydrates and of substances soluble in alcohol, and about 8·5 per cent. of mineral matters. They found no indol and neither leucine nor tyrosine in the intestinal contents which had passed through the whole length of the small intestines.

The facts which we have passed under review bring very clearly before us that as the intestinal contents pass from the ileum into the colon, they yet contain considerable quantities of alimentary constituents which are not only capable of absorption but which, as analyses of the fæces teach us, are actually absorbed in the colon.

Although, as we have seen, processes of fermentation are ripe in the small intestine, they are almost entirely confined to the carbohydrates, and scarcely affect the albuminous substances. The acids, especially lactic acid, developed by the action of the various micro-organisms on the sugars are unfavourable to the action of putrefactive bacteria on the proteids—an action which is essential to the changes which shall convert the unabsorbed intestinal residue into the *fæces*.

SECT. 3. THE FINAL DIGESTIVE PROCESSES IN THE LARGE INTESTINE. ITS POWERS OF ABSORPTION. THE PROCESSES WHICH CONVERT THE CONTENTS OF THE COLON INTO FÆCES.

The secretion of Lieberkühn's glands in the large intestine.

We have seen that the intestinal juice poured out by the glands of the small intestine is characterised by a highly alkaline reaction, which depends upon the presence of sodium carbonate, and by the presence in it of enzymes capable of acting only on certain of the carbohydrates (sugars). In spite, however, of this alkaline reaction of the juice poured out in the small intestine, the contents possess a marked acid reaction, due to the products of fermentation engendered by the action of micro-organisms on the sugars.

The mucous membrane of the colon possesses, according to Nencki, Macfadyen and Sieber, a more powerfully alkaline reaction than that of the small intestine. Presumably its secretion consists of a mucin-containing liquid rich in sodium carbonate. All evidence, as we shall point out, seems to shew conclusively that it contains no enzymes.

The processes of the large intestine which are of importance in so far as the nutrition of the organism is concerned, are chiefly processes of absorption; though, perhaps to a less extent than in the small intestine, processes of fermentation may help to render soluble some fractions of the food constituents which had remained unacted upon.

Not only does the large intestine possess the power of absorbing the soluble albumins, the peptones, the sugars, and a great part of the water present in the intestinal contents which pass through the ilio-colic valve, but (as first demonstrated by the experience of medical men) it possesses even in its lowest part, the rectum, powers of absorption which enable life to be supported for long periods of time by rectal injections (nutrient enemata), when no food can enter the system by the usual channels.

The researches of Bauer.

In 1869, in a research conducted under Voit's direction, Bauer[1] shewed that if dogs are kept without food for some days, and when the daily excretion of urea has become constant, rectal injections of albuminous substances are made, the amount of urea excreted increases at once, proving that the albumin has actually entered the economy. Having, in a similar manner, injected solutions of so-called peptones (doubtless composed almost entirely of albumoses), Bauer found that these were most readily absorbed; an increase of urea, amounting to eight grammes, occurring. This quantity of urea represented the absorption of 21 grammes of dry albumin or 100 grammes of fresh meat. Curiously, Bauer found that a solution of white of egg was not absorbed, unless mixed with sodium chloride. On the other hand, a solution of syntonin was absorbed as readily, or nearly so, as one of peptones.

The researches of Eichhorst.

In 1871 Professor Eichhorst of Zürich[2], then a student of medicine in Königsberg, published the results of a research, conducted under the direction of Professor von Wittich, which established, 1stly, that the mucous membrane of the large intestine formed neither a diastatic nor a proteolytic ferment: 2ndly, that the large intestine possesses the power of absorbing Meissner's a-, b-, and c- peptones (*i.e.* albumoses and peptones), Liebig's extract of beef, the albuminous bodies of milk, dissolved myosin, dissolved alkali-albuminate, egg albumin mixed with common salt, and solution of gelatin: 3rdly, that it is unable to absorb pure white of egg, syntonin solutions, the albumin of blood serum as well as undissolved fibrin, syntonin and myosin.

The researches of Bauer and of Eichhorst received confirmation and valuable practical application at the hands of Leube[3] as well as of

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[1] J. Bauer, 'Ueber d. Aufsaug. im Dick- und Dünndarm,' *Zeitschr. f. Biologie*, Vol. v. (1869).

[2] Hermann Eichhorst, Cand. Med. aus Königsberg, 'Ueber die Resorption der Albuminate im Dickdarm' (Von der med. Fakultät der Albertus-Universität zu Königsberg mit dem Preise gekrönt), Pflüger's *Archiv*, Vol. IV. (1871), pp. 570—662.

[3] W. O. Leube, 'Ueber die Ernährung der Kranken vom Mastdarm aus,' *Deutsch. Archiv f. klin. Medizin*, Vol. x. (1872), pp. 1—54.

Czerny and Latschenberger[1]. The latter observers from observations made on a case in which a præternatural anus existed in the left inguinal region, communicating with the sigmoid flexure, came to the conclusion that soluble albumin undergoes no change when brought in contact with the human large intestine, but is absorbed as such. Every circumstance which leads to an irritation of the gut hinders absorption. Their experiments led them however to form an estimate that the whole large intestine could only absorb 6 grammes of albumin in the space of 24 hours—a quantity quite insufficient to support the life of man. It seems obvious that the discrepancy between these results and those of other observers is to be explained by the fact that the conditions of the case studied by Czerny and Latschenberger permitted only the rectum to be filled with liquid, and that in all probability absorption goes on much more rapidly in the parts of the colon situated higher up.

In a research of great interest which he performed with the aid of a patient with a præternatural anus communicating with the cæcum, Marckwald, besides shewing that the secretion of the colon possesses neither diastatic nor proteolytic power, determined that the whole large intestine is only capable of absorbing about 250 grammes of water in the course of 12 hours. Curiously, in the case observed by him, solution of albumin did not appear to be absorbed, and peptones gave rise to great irritation[2]. If we consider the non-absorption of albumin and peptones observed by Mackwald by the aid of the information supplied by previous observers, to wit, that all irritation of the large intestine seems to arrest the absorption of dissolved albuminous bodies (not apparently the absorption of NaCl), we shall arrive at the conclusion that his anomalous results were probably due to a morbid condition of the large intestine, existing in his case.

SECT. 4. THE MICRO-ORGANISMS OF THE COLON AND THEIR PRODUCTS. THE CONVERSION OF THE CONTENTS OF THE COLON INTO FÆCES.

When the intestinal contents reach the ilio-cæcal valve they contain no longer either pepsin or trypsin. We have discussed the destruction of the former, and with reference to the latter we can have no doubt that it is gradually destroyed by the organic acids of the small intestine. We have adduced the concordant testimony of several most competent observers who have shewn that no enzymes are formed in or secreted by the mucous membrane of the large intestine. We have now, however, to consider whether micro-organisms exist in the large intestine which possibly subserve a useful function by completing the solution of the undissolved albuminous and carbohydrate constituents of food which have resisted all previous agencies.

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[1] v. Czerny and T. Latschenberger, 'Physiologische Untersuchungen über Verdauung und Resorption im Dickdarm des Menschen,' Virchow's *Archiv*, Vol. LIX. (1874), pp. 161—190.

[2] Max Mackwald, 'Ueber Verdauung und Resorption im Dickdarme des Menschen' (Aus d. phys. Inst. von Prof. Kühne zu Heidelberg), Virchow's *Archiv*, Vol. LXIV. (1875), pp. 505—539.

It was pointed out by Marckwald, in the research to which reference has already been made, that, although no proteolytic enzyme exists in the colon, albuminous matters introduced into it, give rise, under the influence of putrefactive processes, to small quantities of peptones which are doubtless absorbed.

The characteristic micro-organisms of the large intestine.

The researches of Bienstock[1], William Booker[2], Macfadyen, Nencki and Sieber[3], of Jakowski[4] and of Zumft[5], have now placed us in possession of much information as to the micro-organisms of the colon and particularly leave no room for doubt that several exist which exert a powerful action on the carbohydrate constituents.

Bienstock isolated four micro-organisms existing in human fæces, amongst which he found one which he considered to be specially concerned in the decomposition of albuminous substances, *Bacillus putrificus coli.* Booker in general confined his observations to the fæces of suckling children and found them to contain nearly pure cultures of *Bacterium coli commune.* He found this organism to increase when diarrhœa supervened, in proportion to the severity of the disease. He also observed an organism resembling the *Bact. lactis aërogenes.*

Macfadyen, Nencki and Sieber isolated three organisms from the contents of the colon, viz. (1) the *Streptococcus liquefaciens ilei,* closely allied to the *Streptococcus coli gracilis* of other authors: (2) the *Bacterium Bischleri,* doubtless one of the forms of the *Bacterium coli commune:* (3) *Bacterium lactis aërogenes.* All 'bouillon' and gelatin cultures made with the contents of the large intestine possessed a repulsive odour of putrefaction and the majority of the colonies obtained with plate cultures consisted of a non-fluorescing putrefactive bacillus. Jakowski, in a recent research, conducted in Nencki's laboratory at St Petersburgh, isolated the following organisms from the contents of the colon:—*Bacterium liquefaciens coli: Streptococcus coli gracilis: Bacillus putrificus coli* (Bienstock).

Amongst the organisms just mentioned, the *Bacterium coli commune* has an intense action on sugar. It gives rise, according to the observations of Dr Bischler[1], to a most energetic fermentation, in which are formed ethylic alcohol, acetic acid, and dextrogyrous paralactic acid[2]. As was stated in discussing the micro-organisms of the small intestine, the *Bacterium lactis aërogenes* has an intense action on sugar, developing alcohol and according to Frey dextrogyrous paralactic acid. When grown in the absence of air it decomposes sugar with the production of a mixture of gases, containing 72·38 vol. p.c. of CO_2 and 27·61 of H.

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[1] Bienstock, *Zeitschrift f. klin. Med.*, Bd. VIII. (quoted by Macfadyen, Nencki and Sieber, *op. cit.*, p. 336).

[2] William Booker (quoted by Macfadyen, Nencki and Sieber).

[3] Macfadyen, Nencki and Sieber, *op. cit.*, p. 337.

[4] Jakowski, 'Contributions à l'étude des processus chimiques dans les intestins de l'homme' (Travail du laborat. de M. Nencki), *Archives des Sciences Biologiques, St Pétersbourg.* Tome I. (1892), p. 539.

[5] Zumft, see foot-note 3, p. 455.

It is the *Streptococcus liquefaciens coli* and the other putrefactive bacteria to which reference has been made, which are the cause of the putrefactive fermentation which attacks the proteids. That this produces small quantities of peptones and other soluble products of the putrefactive decomposition of albumin, as Marckwald shewed, and that these are absorbed, is likely enough, but the part which they play in reference to the nutrition of the body must be a very limited one.

The action of these organisms is, however, able to convert into fæces the residual matter left after the absorption of a large part of the water and of the soluble constituents which the intestinal contents possessed on entering the colon. The alkalinity of the mucous secretion of the colon is such as more than to neutralise the organic acids which are the products of the action of the *bacterium commune coli*, &c., and the alkaline reaction, which is so favourable to true putrefaction, is established.

The putrefactive process, which attacks the residues of the digestive process, is one in which the proteid bodies are decomposed with the production of characteristically stinking products, amongst which skatol is the chief[3]. The nascent hydrogen, which is evolved during its progress, acting on the bilirubin, which had retained its individuality, now converts it into hydrobilirubin, the characteristic colouring matter of the fæces. Yet, even when the putrefactive changes are complete, the fæces have, under normal circumstances, to sojourn a while in the rectum, where the absorption of water and perhaps even of other diffusible substances goes on until they are expelled from the body.

It is a strange, and a somewhat puzzling, fact that the hydrobilirubin, the indol, the skatol, the phenols, which are the result of the putrefactive process, which goes on in preponderating measure, if not exclusively, in the large intestine, should in great measure be absorbed and, after entering the portal blood and making their way through the liver, be excreted, somewhat modified or in new combinations, in the urine. That these bodies play a part in influencing the metabolic processes of the body, scarcely admits of a doubt. The phenols, the indol, even the fœtid skatol, which result from the life-work of the putrefactive bacteria of the intestine, illustrate the general law that the products of living organisms are prejudicial to, and capable of destroying, organisms of the kind which produced them: for all these bodies are antiseptics of more or less power.

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[1] Original observations communicated to Dr Blachstein. See his 'Contribution à la Biologie du Bacille Typhique,' Premier Mémoir, p. 11.

[2] See also A. Baginsky, 'Zur Biologie der normalen Milchkotbakterien,' *Zeitsch. f. phys. Chem.*, Vol. XIII. (1889), p. 353.

[3] Zumft, in a recent research conducted in Nencki's laboratory at St Petersburgh, found no skatol-carbonic acid in the contents of the colon. See Zumft 'Sur les processus de putréfaction dans le gros intestin de l'homme,' &c. *Archives des Sc. Biol., St Pétersbourg.* Tome I. (1892), p. 497.

The absorption of the hydrobilirubin, and its subsequent excretion in modified forms in the urine, may be a mere accident of its solubility and diffusibility, or it may play a real, though yet undiscovered, part.

SECT. 5. THE FÆCES IN HEALTH.

Amount per diem.

The weight of the fæces excreted by healthy men amounts, on an average, to one-seventh or one-eighth of that of the solid food, and may be, therefore, estimated as from 130 to 200 grms. per diem, but the amount varies remarkably with the nature of the food, with the way in which it has been cooked, or otherwise prepared, &c. When the diet is, in the main, a vegetable one containing much cellulose, the amount of unassimilable matter to be got rid of is much larger than when the diet is mainly an animal one. The quicker, too, the passage of the food through the alimentary canal, i.e. the more rapid the intestinal contractions, the greater, *cæteris paribus*, the weight of the fæces.

Colon.

The consistence of the fæces varies greatly and depends, mainly, on the length of sojourn in the colon. The colour is much influenced by the nature of the food, by the abundance or otherwise of the biliary colouring matters and their derivatives, and by the accidental presence of foreign elements, especially of a metallic nature. Thus the fæces passed on a purely animal diet are dark brown, and on a milk diet are yellowish white. When iron and bismuth are present, even in small quantities, in the stools, the colour is a black one, due to the formation of sulphide of iron. After the administration of calomel, the stools have a green colour, which was formerly supposed to be due to an admixture with sulphide of mercury. The true explanation appears, however, to be that under the influence of calomel intestinal putrefaction is arrested and the biliverdin which is derived from the oxidation of the bile colouring matter passes unchanged into the stools. When the bile is cut off from the intestine the stools are clay coloured.

The amount of solid matters in the fæces varies between 17·4 and 31·7 p.c.

The following results were obtained by Bischoff and Voit in the case of carnivores.

"When fed upon a purely flesh diet a strong dog excreted in 24 hours only 27—40 grms. of fæces containing, on an average, 12·9 grms. of solids, though the amount of meat consumed varied between 500 and 2500 grms. Under these conditions, the fæces are almost black, tenacious like pitch or simply solid and are only evacuated at intervals of some days. On a diet of bread, defæcation occurs at least once daily, and the weight of fæces excreted is much greater than on a flesh diet, reaching one-sixth to one-eighth of the weight of the food ingested. Thus in Bischoff and Voit's first set of experiments, the amount of bread consumed per diem amounted to 857 grms., which corresponded to 460 grms. of water-free bread. The fæces weighed 377 grms. and contained 76 grms. of solid matters, so that for every 100 grms. of bread consumed, there were excreted 16·6 grms. of fæces. Fæces passed on a purely bread diet are of a yellowish-brown and crumble easily. They possess a strongly acid reaction and are coloured of an intense blue by iodine. The percentage composition of these fæces, compared with that of bread, shews that they are composed of nearly unchanged bread which the digestive apparatus has been unable to utilise, whilst the fæces passed on a flesh diet differ widely in composition from flesh, as the subjoined table will shew[1].

Results of Observations on the Dog (Bischoff and Voit).

	Bread	Fæces excreted on a purely bread diet	Meat	Fæces excreted on a purely meat diet
C per cent.......	45·51	47·39	51·95	43·44
H	6·45	6·59	7·18	6·47
N	2·39	2·92	14·11	6·50
O	41·53	36·08	21·37	13·58
Mineral matters	4·12	7·02	5·39	30·01

Reaction of the fæces. We have drawn attention to the almost universally propagated error that the contents of the small intestine usually possess an alkaline reaction. We have now to emphasize the parallel error which is also widely diffused, viz. that the contents of the large intestine and the fæces possess an acid reaction. The exact contrary is true. Usually the normal fæces of man are alkaline, and it is very exceptional that they present an acid reaction.

The odour of the fæces. The specific foul odour of fæces is due to indol and especially to skatol, developed by the action of putrefactive bacteria in the colon. Occasionally, sulphuretted hydrogen, ammonia, and other volatile bases, contribute to the fœtid odour.

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[1] This account of Bischoff and Voit's experiments (*Die Gesetze der Ernährung des Fleischfressers*, Leipzig u. Heidelberg, 1860, p. 290) is taken from the paper by Macfadyen, Nencki and Sieber, *Archiv f. exp. Path. u. Pharm.*, Vol. xxviii. (1891), p. 344.

Microscopic characters of the fæces.

On microscopical examination, the fæces of man and animals consuming a mixed diet may exhibit the following structures: vegetable parenchyma, starch granules, spiral vessels, masses of woody fibre, fragments of muscular fibres, fragments of tendon and ligament, yellow elastic fibres, fragments of blood-vessels, masses of fat, crystalline calcium salts of the fatty acids, undissolved nucleins, besides mucus and epithelium which appears to be abundantly exuviated by the intestinal walls. There are also frequently found chlorophylloid matters, crystals of ammoniaco-magnesium phosphate &c.

The derivatives of bile colouring matter found in the excrements. Hydrobilirubin.

The brown colour of the normal fæces is due to hydrobilirubin. Heynsius and Campbell[1] had expressed the opinion that the colouring matter of the fæces was identical with choletelin. Vanlair and Masius[2] afterwards described the colouring matter of the fæces as a body very closely resembling choletelin in properties, especially in spectroscopic characters, but which they considered distinct from it, assigning to it the name of '*Stercobilin*.' Jaffé looked upon the colouring matter of the fæces as identical with the urobilin of the urine[3]. Maly[4], however, subsequently shewed that the colouring matter of the fæces is doubtless the same as the product of reduction which he had obtained from bilirubin and to which he had assigned the name 'hydrobilirubin'. The origin of the latter body from bilirubin, under the influence of the nascent hydrogen, abundantly evolved during intestinal digestive processes, sufficiently explained its occurrence. Notwithstanding the arguments of MacMunn, which are based on minor differences in spectroscopic reactions, the Author is of the opinion, which is shared by nearly all physiological chemists, that the normal colouring matter of the fæces is a product of reduction and is identical with Maly's hydrobilirubin.

The derivatives of the bile acids found in the fæces.

Some undecomposed glykocholic acid has been found, by Hoppe-Seyler, in the excrements of the ox. In the main, however, as has already been repeatedly insisted on, the greater part of the bile acids are removed by absorption, and only small quantities of cholalic and choloidic acids are found in the fæces. It is remarkable that the fæces only contain very small quantities of the extremely resistant taurine. Dressler[5], by calculating the sulphur existing in organic combination in the fæces as taurin, found that the total quantity excreted in 24 hours only amounted to 0·32 grms. This fact affords an additional proof that the bile acids are mainly reabsorbed in the alimentary canal.

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[1] Heynsius and Campbell, *op. cit.*, p. 320.

[2] Vanlair and Masius, 'Neuer Abkömmling des Gallenfarbstoffs im Darminhalt,' *Centralbl. f. d. med. Wissenschaft*, 1871, no. 24.

[3] Max Jaffé, 'Vorkommen von Urobilin im Darminhalt,' *Centralblatt f. d. med. Wissenschaft*, 1871, no. 30.

[4] Richard Maly, 'Künstliche Umwandlung von Bilirubin in Harnfarbstoff,' *Centralblatt f. d. med. Wissensch.*, 1871, no. 54. 'Umwandlung von Bilirubin in Harnfarbstoff,' Maly's *Jahresber.*, Vol. II. (1874), p. 233 *et seq.* See p. 237.

[5] W. Dressler, 'Beitrag zur Kenntniss der excrementiellen Taurin- und Schwefelausfuhr beim Menschen,' *Prager Vierteljahrsschrift*, Vol. LXXXVIII. (1865), p. 1.

Fats and cholesterin in the fæces.

1. *Fats.* Even the normal fæces of men on a mixed diet contain *small quantities* of the neutral fats, the quantity of palmitin and stearin being said to be greater than that of the olein. When large quantities of fats, especially of oils, are added to the diet, the fæces always contain an excess of fat. Berthé[1], in experiments upon himself, found that when consuming daily a diet composed of 350 grms. of meat, 500 of bread, 60 of fat and 100 of fruit, the quantity of fat daily excreted in the fæces amounted to 7—8 grms. On adding 60 grms. of cod-liver oil to this diet, he excreted, on the 1st day 8 grms., on the 7th day 12 grms., on the 12th day 18 grms., on the 20th day 22 grms., and on the 30th day 49 grms. From this experiment it would appear that, at first, the alimentary canal was able to absorb nearly the whole of the extra fat, but that the capacity of absorption became gradually impaired.

The fæces always contain magnesium and calcium salts of the fatty acids, which are often found crystallised. If the fæces be thoroughly extracted with alcohol and with ether, so as to remove the normal fats and the free fatty acids, and the insoluble residue be then treated with hot acidulated alcohol, the fatty acids, previously in combination with the alkaline earths, pass into solution.

Lecithin is either absent, or present in traces, in the fæces.

2. *Cholesterin.* The normal fæces always contain some cholesterin. According to Austin Flint, jun.[2], the fæces do not contain cholesterin, but a body derived from it to which he gave the name of *stercorin*. It is the universal opinion that Flint's stercorin was merely impure cholesterin.

Excretin and excretolic acid.

In 1857 Marcet[3] separated from the fæces of man a body crystallising in shining needles, soluble in alcohol and ether, insoluble in water, to which he gave the name of excretin, and assigned the formula $C_{78}H_{156}SO_2$. Hinterberger[4] afterwards made an elaborate investigation of this body and, by recrystallising it many times, obtained it free from sulphur. From 100 pounds of excrements he obtained 8 grms. of excretin. According to this investigator, excretin has the empirical formula $C_{20}H_{36}O$. Unlike cholesterin it separates in warty or spherical masses and is less soluble in glacial acetic acid than cholesterin. Excretin when treated with bromine yields a body having the composition $C_{20}H_{34}Br_2O$. By the name of 'excretolic acid,' Marcet described an oily body which he separated from human fæces. These he extracted with hot alcohol and precipitated with calcium hydrate. He decomposed the calcium precipitate with sulphuric acid and then shook with ether, &c. The purified residue had a melting point between 25°—26° C., was insoluble in water, soluble in ether, and easily soluble in hot alcohol. Presumedly, excretolic acid, the product obtained by Marcet, was an impure mixture of fatty acids.

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[1] Berthé, quoted by Maly. Hermann's *Handbuch*, Vol. v. i. p. 243.

[2] Austin Flint, Jun., *Recherches expérimentales sur une nouvelle fonction du foie &c.* Paris, 1868.

[3] W. Marcet, 'On the immediate principles of human excrements in the healthy state,' *Philosophical Transactions*, Vol. CXLVII. Part 1 (1857), pp. 403—413. Also *Annales de chimie et de phys.*, Vol. LIX. (1860), p. 91.

[4] F. Hinterberger, 'Ueber das Excretin,' *Ann. d. Chem. u. Pharm.*, Vol. CLXVI. (1873), p. 213.

Mineral matters found in the fæces.

The fæces contain mineral salts, of which the amount varies greatly according to the nature of the food ingested; these are composed mainly of phosphates of the alkaline earths, with a small quantity of phosphate of iron, silica, &c. The amount of the mineral matters varies between 1 and 8 per cent.

Results of quantitative analyses of Human Fæces.

The water and volatile matters vary between	82·6—68·3 p.c.
The solid matters (organic and mineral).	17·4—31·7 p.c.
Total solid matters excreted in 24 hours	16—57 grms.
Average	30 grms.
100 parts of dried fæces yield on an average	
Matters soluble in ether (mainly fats) .	11·5 p.c.
„ „ alcohol . . .	15·6 p.c.
„ „ water . . .	20·0 p.c.[1]

According to Enderlin[2], the following represents the composition of the mineral matters of the fæces.

Salts soluble in water	Sodium chloride and sulphate	1·37	4·00
	Sodium phosphate	2·63	
Salts insoluble in water	Earthy phosphates	80·37	94·93
	Ferric phosphate	2·09	
	Calcium Sulphate	4·53	
	Silicic acid	7·94	

Porter[3], in a research conducted in the Giessen laboratory, under Liebig, found the mineral matters of human fæces to amount, on the average, to 6·7 p.c. The mineral matters of the fæces passed during 4 days weighed 11·47 grms. The fæces of babies fed only on human milk have been analysed by Wegscheider, who, as the mean of three analyses, found

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[1] Wehsarg quoted by Maly. Hermann's *Handbuch*, Vol. v. I. p. 246.

[2] Enderlin, *Ann. d. Chem. u. Pharm.*, Vol. XLIX. (1844), p. 338.

[3] J. A. Porter, 'Untersuchung der Asche menschlicher Excremente,' *Ann. d. Chem. u. Pharm.*, Vol. LXXI. (1849), p. 109.

Water	in 100 parts	85·13
Organic matters	„	13·71
Mineral matters	„	1·16.

The nature and amount of the constituent organic matters is exhibited by the following mean of 10 analyses:—

Mucin, epithelium and lime soaps in 100 parts	5·39
Cholesterin	0·32
Fats and fatty acids	1·44
Alcohol extractives	0·82
Water	5·35
Inorganic salts	1·36

The Meconium.

This term is applied to the contents of the large intestine of the fœtus, which are expelled at, or after, birth.

Physical characters. The meconium is a dark greenish brown, pitch-like substance, devoid of putrefactive odour; it usually possesses an acid reaction. It exhibits, under the microscope, innumerable cylindrical epithelial cells, united together and often retaining the form of the villi, from which they have become detached. These cells are usually stained of a green colour. Besides epithelial cells, large numbers of cholesterin plates and fat globules are to be seen, as well as crystals of bilirubin.

Chemical characters. The meconium contains from 20 to 28 p.c. of solid matters. These include mucin, bilirubin, biliverdin and bile acids, cholesterin, and small quantities of fats and fatty acids. Zweifel[1], who has made the most complete modern researches on the meconium, as well as Hoppe-Seyler, found neither lecithin nor hydrobilirubin in it. The latter fact affords evidence that no reduction processes occur in the fœtal intestines.

From the meconium of the calf, Hoppe-Seyler[2] obtained as much as 1 p.c. of pure bilirubin. Besides bilirubin, the meconium contains much biliverdin and a colouring matter which exhibits a narrow absorption band on the red side of, and near to, *D*, and a broader and darker band between *D* and *E*. This colouring matter passes into the ethereal solution when calf's meconium is treated with alcohol, the alcohol distilled off and the residue is treated with ether. The ethereal solution possesses a purple-red colour.

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[1] Zweifel, 'Untersuchungen über das Meconium,' *Archiv f. Gynäkologie*, Vol. VII. (1875), p. 474.

[2] Hoppe-Seyler, *Physiolog. Chem.*, p. 346.

The following analyses exhibit the general composition of human meconium (Zweifel).

		(1)	(2)	(3)
Water	in 100 parts	79·78	80·45	—
Solid matters	„	20·22	19·55	—
Cholesterin	„	0·797	—	—
Fats	„	0·772	—	—
Mineral matters	„	0·978	0·87	1·238

In the mineral matters, Zweifel found much sulphuric acid, in combination with calcium and with sodium. He found in the ashes between 1·7 and 3·4 p.c. of $FePO_4$.

SECT. 6. THE FÆCES IN DISEASE.

Fæces in jaundice.

We have already referred to the fact that when bile is cut off from the intestine, the fæces assume a clay colour, are devoid of derivatives of bilirubin (hydrobilirubin), exhale an exceedingly fœtid putrefactive odour, and contain a large quantity of fat.

Fæces in disease of the pancreas.

When the pancreatic duct is occluded, as by cancer of the head of the pancreas, the stools also contain a large quantity of fat. Cases in which the bile-duct is not at the same time pressed upon are rare, so that the opportunity of studying the effects of an uncomplicated occlusion of the pancreatic duct seldom occurs.

The fæces after purgative medicine.

Many purgative medicines appear to act merely by increasing intestinal peristalsis. If the rate of passage of the intestinal contents be increased, it is evident that the absorption of water, and other constituents capable of being absorbed, will be diminished and that the fæces will assume a much less solid character than is normal. On the other hand, the saline and so-called hydragogue purgatives appear to act both by increasing peristaltic action and by augmenting the intestinal secretion (Moreau, Vulpian, Brunton, Matthew Hay); the saline purgatives also act by impeding the absorption of water from the alimentary canal (M. Hay, Röhmann[1]).

In the case of many purgative substances it would appear that, as in diarrhœa due to specific poisons, the intestinal mucous membrane is irritated, an excessive amount of epithelium is thrown off and a pathological transudation from the blood-vessels occurs, as evidenced by the albumin which the liquid dejecta hold in solution.

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[1] For systematic information on this subject, which does not strictly come within the scope of the present work, consult Harnack, *Lehrbuch der Arzneimittellehre und Arzneivorordnungslehre*, Hamburg und Leipzig, Verlag von Leop. Vois, 1883, see pp. 42—45, and Brunton's *Pharmacology, Therapeutics and Materia Medica*. The following papers should be referred to on this subject. Radziejewski, 'Zur physiologischen Wirkung der Abführmittel,' *Archiv f. Anat. u. Phys.*, 1870, p. 1. Lauder Brunton, 'On the action of purgative medicines,' *The Practitioner*, 1874. Dr Matthew Hay, *Journal of Anatomy and Physiology*, Vol. xvi., pp. 243 and 391.

C. Schmidt found the liquid dejections of man, after administration of infusions of senna, to have the following composition[1]:

Water in 1000 parts	969·75
Solid matters	30·25
Albumin	1·64
Other organic matters	20·03
Inorganic salts	8·58

The analysis of the mineral constituents made by this eminent analyst exhibit clearly enough that the secretion was not normal intestinal juice, the solid constituents of which, as we have shewn, contain one-third of their weight of sodium carbonate.

Schmidt found the inorganic salts, referred to in the above analysis, to have the following composition.

K_2SO_4	0·667
KCl	2·680
$NaCl$	2·056
Na_3PO_4	0·658
Na_2O	1·960
$Ca_3(PO_4)_2$	0·325
$Mg_3(PO_4)_2$	0·233

Method of examining the stools in diseases associated with diarrhœa.

In examining the liquid stools passed in diarrhœa depending upon simple intestinal catarrh, typhoid fever, or cholera, the observer should not merely satisfy himself by simple inspection, but should decant the stool into a tall cylinder and examine both the deposit and the supernatant liquid. "In all cases where the stools have to be examined, the noisome odour may be reduced to a minimum by pouring over them (whether liquid or solid) a thin layer of ether. After the sediment has been deposited, the observer can form at a glance an approximate estimate of the amount of water, blood, mucus, and solid matters. He is able to recognize the amount and size of fibrinous exudations and can easily obtain the different constituents of the fæces for microscopic examination. The latter by revealing the presence of pus and blood-corpuscles, of exuviated epithelia, of mucus and the histological elements of tumours, permit of an approximate conclusion being drawn as to the nature and intensity of the pathological processes going on in the intestine[2]." It need scarcely be added that at the present time a bacteriological examination of the fæces in disease is necessary for their thorough scientific investigation.

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[1] C. Schmidt, 'Zur Charakteristik d. epidem. Cholera, &c.' Dorpat u. Mitau (1850).

[2] Freely translated from Ewald—*Die Lehre von der Verdauung. Einleitung in die Klinik der Verdauungskrankheiten. Zwölf Vorlesungen gehalten, vor Aerzten und älteren Studirenden, im Wintersemester* 1878—79, von Dr C. A. Ewald. Berlin, Hirschwald, 1879, see pp. 96 and 97.

The stools in typhoid fever. In typhoid fever, the diarrhœa probably depends partly on diminished absorption of water and partly upon the transudations which result from the inflammatory process set up by the products of the specific organisms which are the cause of the disease. The stools may contain blood and necrotic portions of the mucous membrane. They are said often to contain ammonium carbonate and to exhibit under the microscope crystals of ammoniaco-magnesium phosphate. According to Brieger, they contain no skatol.

The fæces of typhoid fever contain the characteristic bacillus, *bacillus typhi*, first accurately described by Gaffky[1]. The researches of Brieger[2] shew that this bacillus, like the *bacterium coli commune*, decomposes sugar with the production of lactic acid. Whilst however this latter bacterium yields dextrogyrous acid (viz. sarcolactic acid), the bacillus of Gaffky yields lævogyrous paralactic acid (Blachstein[3]). In the first edition of this book three isomeric lactic acids were described, viz. ethylene-lactic acid, and the two ethylidene-lactic acids, to wit, the optically inactive ethylidene-lactic acid or lactic acid of fermentation, and the optically active *dextrogyrous* ethylidene-lactic acid or sarcolactic acid. This, which was also called paralactic acid, may now with convenience be called dextrogyrous paralactic acid. In 1890, Schardringer succeeded in obtaining (as a result of the fermentation of cane sugar by a bacillus, discovered accidentally in water) the hitherto unknown lævogyrous ethylidene-lactic acid, which forms salts which are dextrogyrous (sarcolactic acid, on the other hand, being dextrogyrous forms lævogyrous salts).. So far as is at present known, the only *pathogenic* organism which produces lævogyrous lactic acid, is according to Blachstein, Gaffky's *Bacillus typhi*. From cultures of the bacilli of typhoid fever, Brieger[4] separated a poisonous base, to which he assigned the name *typhotoxin* and the formula $C_7H_{17}NO_2$. When injected into guinea-pigs and mice, typhotoxin produces a lethargic condition, paralysis, and fatal diarrhœa. In addition, active toxalbumins appear to be produced in cultures of the typhoid bacillus (Brieger and Fränkel).

The stools in cholera. In Asiatic cholera, the fæces assume the character of the so-called 'rice-water stools,' *i.e.* they present the appearance of a turbid, flocculent liquid, from which bile is absent, in which the microscope reveals the presence of a large amount of epithelium shed by the intestinal villi, and bacteriological analysis the presence of the cholera spirillum. The liquid fæces contain very little organic solid matter and a considerable proportion of salts. They invariably contain albumin. They exhale a semen-like odour believed to be due to pentamethylendiamin. The following are two analyses of cholera stools made by C. Schmidt:

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[1] M. Gaffky, *Mittheilungen aus dem Kaiserl. Gesundheitsamte*, Vol. II., quoted by Blachstein.

[2] Brieger, *Ueber Ptomaïne*, Th. III. (1886), p. 81.

[3] Dr Blachstein, 'Contribution à la biologie du bacille typhique' (Travail du laborat. de M. Nencki à l'Institut Impérial de Médecine Expérimentale), Num. 1 and 2, Extraite des *Archives des Sciences Biologiques*, Vol. I., St Petersburg, 1892.

[4] Brieger, 'Zur Kenntniss der Bildung von Ptomaïnen und Toxinen durch pathogene Bacterien,' *Sitzungsber. d. Berl. Akad. d. Wissenschaft.* Jan. 1889.

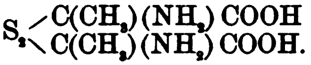
	(1)	(2)
Water in 1000 parts	988·17	985·13
Organic matters	2·99	7·32
Mineral matters	8·84	7·55

Recent investigations have in the case of cholera established certain facts which possess great interest.

Cholera cultures always contain indol and nitrites. The consequence of this admixture is that when dilute sulphuric acid is added to them, or to cholera stools, a red colouration is produced, which is due to the sulphuric acid setting free the nitrous acid of the nitrites, which reacting on the indol present forms of nitrate of nitroso-indol (see page 423)[1]. In cholera cultures, Brieger[2] has found, in addition to the non-poisonous pentamethylendiamin and tetramethylendiamin, the highly poisonous *methyl-guanidin*. Brieger and Fränkel[3] have also found certain toxalbumins which appear to belong to the class of globulins. From these researches it appears probable that certain of the gravest symptoms of cholera are due to the actual poisonous action induced by the chemical products which result from the action of the cholera bacillus on the albuminous matters.

The stools in dysentery. In dysentery, the stools are always intensely fœtid, sometimes of gangrenous odour, and are characterised by the presence of mucus, streaked or stained with blood, and often contain purulent sloughs. So far as the Author knows, the bacteriological study of dysentery and an examination of the chemical products of the pathogenic organisms which cause it, have not yet been made.

The stools in cystinuria. In a future volume, we shall have to study at length 'cystine,' a remarkably interesting sulphur derivative of lactic acid, which occurs in traces in the healthy organism, but which, in certain individuals, is excreted in the urine in considerable quantities and sometimes forms cystine calculi. Cystine was first discovered as a constituent of a very rare form of urinary calculi by Wollaston. It has the empirical formula $C_6H_{12}N_2S_2O_4$. The researches of E. Baumann[4] leave no doubt that cystine is a dithioamido-æthylidene-lactic acid, which may be represented by the subjoined formula:



$$S_2\begin{array}{l}\diagup C(CH_3)(NH_2)\,COOH\\ \diagdown C(CH_3)(NH_2)\,COOH.\end{array}$$

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[1] E. Salkowski, 'Ueber das *Cholerarot* und das Zustandekommen der Cholera-reaktion,' Virchow's *Archiv*, Vol. cx. (1887), p. 360.

[2] L. Brieger, 'Zur Kenntniss d. Bildung von Ptomaïnen und Toxinen durch pathogene Bakterien,' *Sitzungsber. d. Berl. Akad. d. Wissen.*, Jan. 1889.

[3] L. Brieger and C. Fränkel, 'Untersuchungen über Bacteriengifte,' *Berl. klin. Wochensch.*, 1890, pp. 241—246 and pp. 268—271.

[4] Baumann, *Zeitsch. f. physiol. Chem.*, Vol. viii. (1883), p. 300; Vol. xii. (1888), p. 261; Vol. xvi. (1892), p. 552.

In individuals suffering from the cystine-diathesis, the quantity excreted in the urine is comparatively large, as much as one-fourth of all the sulphur which should be excreted in the oxidised condition passing out of the system as cystine. There can be no doubt that this depends upon an abnormal decomposition of the proteids, and facts render it probable that this is dependent on the intervention of abnormal fermentations in the alimentary canal. Neither the fæces nor the urine of healthy human beings contain any trace of diamines (so-called *ptomaines*). Baumann and Udransky[1], as well as Brieger and Stadthagen[2], have found that when cystine occurs in the urine, both the fæces and the urine contain cadaverin, putrescin and another diamine, which is isomeric with pentamethylen-diamine or cadaverin (perhaps neuridin or saprin). It therefore seems very probable that the cystine diathesis depends upon an abnormal ferment process, having its seat in the intestine and due to yet unknown micro-organisms.

SECT. 7. THE GASES OF THE LARGE INTESTINE.

The large, like the small intestines, are always more or less distended by a mixture of gases which, in part, have diffused out of the blood, but in part are the products of the action of micro-organisms on carbohydrates and to a less extent on proteids. The evolution of gases is, however, in the normal condition, a very limited one. An analysis of the gases obtained from the rectum shews them to be composed of a mixture of carbonic acid, marsh gas, nitrogen, and hydrogen. In the strictly normal condition of digestion, unless substances are consumed in considerable quantities which either contain free sulphur, or easily decomposed sulphur compounds, the gases of the large intestine contain no sulphuretted hydrogen.

Origin of the individual gases found in the mixed gases of the colon.

The CO_2 is in part derived from the blood, in part the product of the bacterial decomposition of the carbohydrates and proteids. The H is a product, as we have already shewn, of the action of various bacteria on sugars[3], in part it is produced during the putrefaction of albuminous bodies.

The methane (CH_4) is the product both of the fermentation of carbohydrates and proteids (Kunkel)[4]. It is formed in large quantities by the bacterial decomposition of cellulose (Hoppe-Seyler[1], Tappeiner[2], Henneberg and Stohmann[3].)

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[1] E. Baumann and L. v. Udransky, 'Ueber das Vorkommen von Diaminen, sogenannten Ptomaïnen, bei Cystinurie,' *Zeitsch. f. phys. Chem.*, Vol. XIII. (1889), pp. 562—594.

[2] L. Brieger and M. Stadthagen, 'Ueber Cystinurie nebst Bemerkungen über einen Fall von morbus maculosus Werlhofii,' *Berliner kl. Wochensch.*, 1889, no. 16, and Maly's *Jahresbericht*, 1890, p. 453.

[3] The abundant evolution of H and CO_2 during the butyric fermentation is well known; see Victor Paschutin, 'Einige Versuche über die buttersaure Gährung,' Pflüger's *Archiv*, Vol. VIII. (1873), p. 352.

[4] Kunkel, 'Ueber die bei der Pancreasverdauung auftretenden Gase,' abstracted in Maly's *Jahresber.*, Vol. IV. (1875), pp. 274—276.

The nitrogen is in part doubtless a diffusate from the blood, but is certainly in part derived from the bacterial decomposition of the proteids (Hüfner[4], Kunkel[5].)

The following are the results of analyses of the gases of the human colon, made by Ruge.

Results of Analysis of the Gases of the Large Intestine of Man. Ruge[6].

	Milk diet		Meat diet			Vegetable diet (leguminous diet-pulses)		
	I.	II.	I.	II.	III.	I.	II.	III.
CO_2 in 100 vols.	16·8	9·9	13·6	12·4	8·4	34·0	38·4	21·0
H..................	43·3	54·2	3·0	2·1	0·7	2·3	1·5	4·0
CH_4..............	0·9	——	27·5	27·5	26·4	44·5	49·3	55·9
N..................	38·3	36·7	57·8	57·8	64·1	19·1	10·6	18·9

SECT. 8. INTESTINAL CONCRETIONS[7].

In addition to gall stones, which are very frequently excreted with the fæces *per anum*, or which, remaining impacted in the small intestine occasionally are the cause of fatal intestinal obstruction, intestinal calculi are sometimes met with in the colon of certain of the lower animals, and much more rarely of man. In man, these calculi are usually very small, having a diameter of 0·2—1 mm. (*intestinal gravel*), but occasionally, though rarely, they attain the size of a hazel nut. Intestinal concretions are comparatively frequent in Scotland, owing to the large use made of oatmeal porridge as an article of diet. Such calculi present the appearance of brown balls and resemble, in structure and composition, calculi which are very frequently met with in millers' horses and which, in the latter, often attain an enormous size.

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[1] Hoppe-Seyler, 'Ueber Gährung der Cellulose und Bildung von Methan und Kohlensäure,' *Zeitsch. f. phys. Chemie*, Vol. x. (1886), p. 201 *et seq.* and pp. 401—440.

[2] H. Tappeiner, 'Untersuchungen über die Gährung der Cellulose insbesondere über deren Lösung im Darmcanal,' *Zeitsch. f. Biol.*, Vol. xx. (1884), pp. 52—134.

[3] Henneberg and Stohmann, 'Ueber die Bedeutung der Cellulosegährung für die Ernährung der Thiere,' *Zeitsch. f. Biol.*, Vol. xxi. (1885), pp. 613—624.

[4] Hüfner, 'Ueber ungeformte Fermente und ihre Wirkungen &c.,' *Journ. f. prakt. Chemie*, Vol. x. (1874), p. 1. Maly's *Jahresber.*, Vol. iv. (1875), pp. 262—274.

[5] Kunkel, see Note 4 on preceding page.

[6] Ruge, *Sitzungsber. d. Wien. Akad. d. Wiss.*, Vol. xliv. (1862), p. 734.

[7] In preparing this section the Author has made use of the account given under the heading 'Darmconcremente' at page 174 of Professor Karl B. Hofmann's *Lehrbuch der Zoochemie*, Wien, 1879, as well as of the information contained in Hoppe-Seyler's *Physiologische Chemie*, under the heading 'Darmconcremente' (see p. 356 *et seq.*).

Intestinal concretions in man have often been found to contain some foreign body as a nucleus, as for example a fruit stone, sometimes fragments of bone, of iron, or horn, which have been accidentally swallowed (Hoppe-Seyler). The mineral salts which form the greater part of, or which occur as an incrustation around, intestinal calculi are composed either of pure crystalline ammoniaco-magnesium phosphate, or of this salt mixed with varying quantities of magnesium phosphate.

In certain intestinal concretions of the horse, the amount of triple phosphate has been found to vary between 83·2 and 98·3 per cent., and silica has been found to the amount of 5·2 per cent.

Hair balls.

In certain animals, as the pig, the ox, the goat and the chamois, intestinal concretions are met with, which are composed entirely of felted hairs and which take their origin in the habit of these animals to lick their own coats or those of their associates.

Oriental 'bezoars.'

Oriental bezoars are intestinal concretions probably derived from *Capra œgagrus* and *Antelope dorias*; they are for the most part spherical or oval, possess a dark olive-green colour, and have a smooth, waxy, shining exterior. They vary greatly in size. When heated, true bezoars evolve aromatic fumes.

Lithofellic acid $C_{20}H_{36}O_4$ and the other constituents of true bezoars.

True bezoars are composed almost entirely of an acid called lithofellic acid which was first investigated by Ettling and Will and to which they assigned the formula $C_{20}H_{36}O_4$. Lithofellic acid is extracted from bezoars by powdering and treating with boiling alcohol. On evaporating the alcoholic solution, the latter deposits small colourless crystals which are either pointed rhombohedra or three-sided prisms. Lithofellic acid is insoluble in water, and very sparingly soluble in ether. It is soluble in glacial acetic acid which deposits it in a crystalline condition on evaporation. Lithofellic acid is, according to Hoppe-Seyler, closely related to cholalic acid.

The green colour of bezoars is due to biliverdin; these mysterious concretions contain other derivatives of bilirubin (Hoppe-Seyler).

False Bezoars. Ellagic acid $C_{14}H_6O_8$.

Intestinal concretions, also called bezoars, come from the east, which are of a brownish black colour, which do not melt, and which are composed of ellagic acid ($C_{14}H_{16}O_8 + 2H_2O$), a derivative of tannin. Ellagic acid is found in oak bark. When pure, it is a yellow crystalline powder which, under the microscope, is seen to be composed of yellow prisms occurring singly or in groups. It is soluble in boiling alcohol. Ferric chloride produces a deep blue precipitate. The ellagic acid of bezoars is doubtless derived from the tannin contained in the food consumed by the animals which yield them.

CHAPTER XIII.

CONCERNING THE MODIFICATIONS OBSERVED IN THE CHEMICAL PROCESSES OF DIGESTION IN SOME DIVISIONS OF THE ANIMAL KINGDOM.

THE researches which have of recent years been made by such distinguished observers as the lamented Professor Krukenberg, Professor Metschnikoff and others, on the process of digestion throughout the animal kingdom have been so numerous and so specialised that it would be hopeless to give their results in detail without unduly extending the limits of this work. The following brief notes may, however, prove useful to the student of comparative physiology.

SECT. 1. THE INTRA-CELLULAR DIGESTION OF THE LOWER INVERTEBRATA.

In certain elementary animal forms, each particle of their structure possesses the same nutritive endowments as its fellows, and there is no differentiation of cells set apart to discharge those chemical functions which we term *digestive*, and which consist essentially in the secretion of a juice or juices, whose function it is to render the food constituents soluble and diffusible, so that these can make their way into the interior of the organism and replace the matter which the latter is ceaselessly losing. In the lower organisms to which we refer, digestion is said to be *intra-cellular*.

The simplest and, in many respects, the most typical example of intra-cellular digestion is that presented by certain Rhizopods, which consist essentially of a mass of living contractile, somewhat granular, protoplasm with a nucleus. The earlier knowledge of the phenomena associated with the ingestion and digestion of food by these unicellular organisms rests on the observations of Dujardin, Kölliker, Carter, Haeckel, De Bary and others, whilst we owe the most recent, complete, and interesting facts relating to these processes, as they can be observed in *Amœba proteus* and *Actinosphærium,* to the investigations of Miss Greenwood[1], to some of whose results the attention of the reader will now be drawn. When an Amœba is observed in active movement, it is seen to be progressing in one direction, "so that a fairly constant hind end may be distinguished, and in this organism it is the hind end which most actively ingests. The ectosarc is drawn like a funnel round the prey, and the opening which corresponds to the mouth of the funnel is ex-centrically closed. In the case of quiescent solid matter, such as starch grains or Torulæ, but little fluid is included; when, however, an actively moving prey is dealt with, an area of water not inconsiderable surrounds it. The ectosarc of two enclosing pseudopodia first fuses distally of the object and leaves a space in which movement goes on—a space only gradually reduced, and never done away with by complete inclusion." Some of the lateral pseudopodia of Amœba proteus may exercise prehensile function, but ingestion at the most anterior and most actively moving part never occurs. The length of time occupied by this act of ingestion varies greatly in different protozoa, but in Amœba is comparatively short though variable. 'Contact may be established and broken many times with no result, but determinate ingestion is a matter of minutes rather than hours.' In studying the digestive processes of the Amœba and Actinosphærium, Miss Greenwood supplied the creatures with various kinds of solid particles, viz. with (*a*) starch grains and cellulose; (*b*) fat globules (milk); (*c*) organisms in which a protoplasmic body is surrounded by a cellulose wall; (*d*) unshielded proteïd chiefly in the form of small animals without tests; (*e*) presumedly innutritious matter, such as litmus. Of these substances, the following appeared useless to the Amœba for purposes of nutrition: the fat, the pure carbohydrates and the litmus. If we take, as an example of these, starch grains, we find that after a stay of some days they are ejected unaltered in form, in size, and in their reaction towards iodine. It was mentioned that when solid bodies are ingested by an Amœba, after their enclosure a space (vacuole) containing some water is seen surrounding them. If the body be one which the creature cannot digest, this water soon disappears, and is not replaced by any newly secreted liquid, *i.e.* the *vacuole* ceases to exist. When, however, either naked or 'shielded' protoplasmic food is ingested by the Amœba, its presence seems to act as a stimulus to the secretion of a liquid which surrounds the matter to be acted upon, which may then be said to occupy 'a vacuole of digestion.'

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[1] M. Greenwood, 'On the Digestive Process in some Rhizopods,' (From the Physiological Laboratory, Cambridge), *Journal of Physiology*, Vol. VII. (1885—1886), pp. 253—273. Besides recording the results of her own observation, Miss Greenwood has in this paper given an account of the earlier literature of the subject.

That the liquid which is poured out into a digestive vacuole must actually exert a chemical action upon protoplasmic bodies might be held to be proved by the fact that the latter may be actually seen to disintegrate as it surrounds them. A conclusive proof is, however, afforded by the digestion of organisms possessed of a cellulose wall; the latter is neither dissolved nor perforated, and yet its contents are digested, a result which is only explicable on the supposition that an active digestive liquid has been able to diffuse through the cellular wall and then exert its solvent action.

Engelmann believed that he had obtained evidence that an acid reaction is developed in Infusoria and some species of Amœbæ during digestive activity, and Meissner and Favre-Domergue are said by Neumeister to have obtained similar results[1]. On the other hand Miss Greenwood was unable by means of methyl-violet and tropæolin, to determine the presence of an acid reaction either in Amœba or in Actinosphærium.

The observations of Metschnikoff[2] on the power possessed by the colourless corpuscles of the blood to seize and disintegrate bacteria and on which he founded his view of their function as phagocytes bear the closest resemblance to those which we have been discussing. Without further dwelling upon these, however, attention must be drawn to the fact that Metschnikoff, Ray Lankester[3] and other reliable observers ascribe powers of intra-cellular digestion to the endoderm and mesoderm cells of some comparatively highly differentiated organisms, provided with an alimentary canal[4].

It appears to be a general law that a specialised digestive mechanism, associated with the secretion of specific digestive enzymes, is possessed by no parasitic creature inhabiting the alimentary canal of its host—a law of which examples are furnished amongst Protozoa by the Gregarinæ and amongst Vermes by many Cestodes and by the Acanthocephali.

> "Complete degeneration of the gut is clearly due to adaptations to definite modes of life, in which the food passes through the integument by endosmosis. This phenomenon, brought about by parasitism, attains its highest developement in the sporocyst forms of the Nematoda. Finally the absence of an enteric canal is the rule among the Cestoda, where the enteron is not present for a time even. The enteron is altogether wanting in the Acanthocephali, and for the same reason—namely, parasitism."[5]

That these parasites possess an inter-cellular digestion seems to be proved by their storing glycogen (M. Foster) which they probably produce at the expense of the sugar and the soluble proteids which they derive from their hosts.

If we exclude Rhizopoda, Gregarinæ and Infusoria, all non-parasitic animals belonging to the sub-kingdoms above Protozoa possess a differentiated digestive function, and, with few exceptions, have been found to form digestive enzymes.

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[1] M. Meissner, 'Beiträge zur Ernährungsphysiologie der Protozoen,' *Zeitsch. f. wissensch. Zoologie*, Vol. XLVI. (1888), p. 498 and Favre-Domergue (*Annales des Sciences Naturelles, Zoologie*, 1888, p. 140), quoted by Neumeister (*Lehrbuch, etc.* p. 115).

[2] Elias Metschnikoff, 'Ueber die Beziehung der Phagocyten zu Milzbrandbacillen,' Virchow's *Archiv*, Vol. XCVII. (1884), pp. 502—526.

[3] Ray Lankester, *Quarterly Journ. of Micr. Science*, Vol. XXI. p. 123. The Author has not been able to verify the quotation.

[4] Thus Metschnikoff asserts that the cells of the endoderm of *Mesostomum* and those of the mesoderm of *Synapta* possess ingestive and digestive power, see M. Greenwood, *op. cit.* p. 257 and Metschnikoff, 'Ueber die Verdauungsorgane einiger Süsswasserturbellarien,' *Zool. Anzeiger*, 1878, p. 387 and 'Untersuchungen über die intracellulare Verdauung bei wirbellosen Thieren.' *Arbeit d. zoolog. Inst. in Wien*, Vol. V. (1883), Heft 2.

[5] Carl Gegenbaur, *Elements of Comparative Anatomy*, translated by Professor F. Jeffrey Bell, M.A. The translation revised and a preface written by Professor E. Ray Lankester, M.A., F.R.S. London, Macmillan and Co., 1878. Refer to pages 158 and 159.

According to Krukenberg[1], the Hydro-medusæ and the sea-Anemones, although possessed of differentiated alimentary apparatus, form no secretion possessed of digestive properties. The external slimy secretion of certain of these creatures in some cases possesses active irritating properties, and appears to subserve defensive purposes, but is destitute of digestive enzymes.

SECT 2. THE FUNCTION OF THE SO-CALLED 'LIVER' OF MOLLUSCA.

The function of the 'mid-gut gland' or liver of Mollusca.

In connection with the mid-gut of the Mollusca there is developed, by the sacculation of the endoderm, a lobulated glandular organ, which opens into the enteron. This organ, which has usually been termed by comparative anatomists the liver (sometimes the 'hepato-pancreas'), and which appears to be the morphological homologue of the organ bearing that name in Vertebrata, discharges functions which are altogether unlike those of the liver. It is destitute of either of the specific products—bile-colouring matters and bile-acids—which are characteristic of the secretory activity of the hepatic cells; but contains several enzymes which confer upon it powers of digesting the various groups of alimentary principles.

From the researches of Krukenberg[2] it results that this gland secretes a diastatic, a fat-splitting and either a pepsin-like or a trypsin-like enzyme. In some molluscs as in *Helix pomatia*, which possess an acid intestinal digestion, the mid-gut gland forms a ferment like pepsin but secretes no trypsin, whilst in other molluscs no pepsin is present, but trypsin. A. B. Griffiths[3] has compared the secretion of the 'liver' of Cephalopoda with that of the pancreas, and has obtained results which prove the identity of function in so far as the creatures he investigated were concerned. In Cephalopoda the secretion is alkaline, converts starch into sugar, emulsifies fats, and dissolves proteids.

Though not discharging the functions of the liver as a bile-secreting gland, it is conceivable that the mid-gut gland of Mollusca might fulfil another function which the liver of vertebrates possesses—that of a storer of reserve material, of a former and hoarder of glycogen. The painstaking researches of Levy[4] have shown, however, that although glycogen is a constituent of the gland, it is present in smaller proportion than in the other organs. He discovered, however, a very small quantity of 'sinistrin,' a dextrin-like carbohydrate first described by Schmiedeberg[1] as present in the bulb of squill (*Urginea scilla*), which does not yield a sugar when digested with diastase, but when boiled with dilute sulphuric acid yields lævulose and an inactive sugar.

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[1] C. Fr. W. Krukenberg, *Grundzüge einer vergleichenden Physiologie der Verdauung.* Heidelberg (Carl Winter), 1882.

[2] Krukenberg, *Grundzüge, etc.* pp. 59 and 61.

[3] A. B. Griffiths, 'Chemisch-physiologische Untersuchungen über die Cephaloden-Leber und ihre Identität mit einem wahren Pankreas,' *Ber. d. deutsch. chem. Gesellsch.* Vol. XVIII. (1885), p. 294. This is a short abstract of the original paper in the *Chemical News*, Vol. LI. p. 160, which the Author has not had access to.

[4] Max Levy, 'Zoochemische Untersuchungen der Mitteldarmdrüse von Helix pomatia,' *Zeitsch. f. Biologie*, Vol. XXV. (1890), p. 410.

SECT. 3. SOME PECULIARITIES OF THE DIGESTIVE PROCESS IN FISHES.

In fishes, the salivary glands are either absent or rudimentary, though Krukenberg found[2] that the buccal mucous membrane of the carp and of *Lophius piscatorius* secreted a fluid possessed of diastatic activity. The stomach secretes a very active gastric juice which was supposed (like that of the frog) to contain a pepsin somewhat differing from that of warm-blooded animals, in that it possessed digestive activity at a temperature of 0° C.[3] The experiments of Krukenberg and of Max. Flaum[4] have shewn, however, that the ability to effect proteolysis at 0° C. is connected with the presence of a large *quantity* of pepsin, and does not imply a difference between the pepsin of warm- and cold-blooded animals.

The striking peculiarity of a large number of fishes is the absence of a definite pancreas; in many of these there exist, however, the so-called *appendices pyloricæ*, cæcal tubes connected with the pyloric portion of the mid-gut, obviously the homologues of the mid-gut gland of Mollusca, and discharging the same function, and in some fishes so held together by connective tissue and united at a common efferent duct as to have the appearance of a compact gland[5]. There are fishes, however, in which both pancreas and *appendices pyloricæ* are absent; in these cases the mucous membrane of the mid-gut secretes a juice which is possessed of diastatic and proteolytic (trypsin) activities[6].

SECT. 4. SOME PECULIARITIES OF THE DIGESTION OF BIRDS.

"In the fore-gut of Birds there is a great division of labour. The influence of adaptation to the mode of life, and more especially to the mode of nutrition, is most clearly shewn by the variations in the different arrangements. The œsophagus, which is of the same length as the neck, is either of equal calibre along its whole course, or is provided with a widened portion, or with a cæcal diverticulum, which looks like an appendage. Portions of this kind, which are characterised by modifications of the glandular organs of the mucous membrane, form a crop (*ingluvies*). This is best developed in carnivorous and graminivorous birds; in the former, indeed, it generally forms a spindle-shaped enlargement, while in the latter it forms a unilateral diverticulum, which is differentiated into a cæcal appendage, in many provided with a narrow connecting piece."

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[1] O. Schmiedeberg, 'Ueber ein neues Kohlenhydrat,' *Zeitsch. f. phys. Chem.* Vol. III. pp. 112—133.

[2] Krukenberg, *op. cit.* p. 67.

[3] Fick and Murisier, 'Ueber das Magenferment kaltblütiger Thiere,' *Arb. a. d. physiol. Lab. d. Würzburger Hochschule*, 1872, p. 181.

[4] Maximilian Flaum, 'Ueber den Einfluss niedriger Temperaturen auf die Functionen des Magens' (Aus d. physiolog. Inst. d. Universität Bern), *Zeitsch. f. Biologie*, 1892, pp. 433—449.

[5] Gegenbaur, *op. cit.* p. 560.

[6] Ralph Blanchard, 'Sur les fonctions des appendices pyloriques,' *Comptes Rendus*, Vol. XCVI. (1883), p. 1241.

"The next portion of the œsophagus, which is generally narrower, passes into the stomach, in which two divisions can be made out; the first is known as the *proventriculus*[1]; its walls are generally thickened by a glandular layer. The second is characterised by the great development of its muscular layer, the strength of which varies with the mode of life of the animal. Where it is greatly developed we may observe a tendinous disc on either side. In the Raptores, as also in many Natatores that live on animal food, the muscular layer is feebly developed. It is very strong in the graminivorous forms (Gallinæ, Anatinæ, Columbæ, Passeres). This portion, which serves for the comminution of food, and compensates for the absence of masticatory organs, may be provided with other arrangements also which serve the same purpose; its inner surface may be covered by a firm horny layer, which is often of considerable thickness, and functions as a radula[2]."

Full of interest as are the processes of digestion in birds, our knowledge of them is derived almost entirely from the writings of the great naturalists of the past, and is in very few particulars dependent on the researches of the moderns. It is to Borelli, Redi, Spallanzani, Réaumur and John Hunter that we are mainly indebted for it.

The function of the 'ingluvies,' or 'crop.' This diverticulum of the œsophagus exists in its most perfectly-developed form in graminivorous birds. The grain and other food which these birds swallow first enters the crop, which becomes distended with it, and which secretes a fluid which moistens its contents. According to Tiedemann and Gmelin, the contents of the crop often present an acid reaction.

So far as the Author is aware, no modern investigation has been made with the object of determining the precise chemical characters of the liquid secretion of the crop, and specifically to ascertain whether it contains any digestive enzymes. The sojourn of food in it is a long one, though it varies within wide limits. Tiedemann and Gmelin noticed that grains swallowed by a hen remained in the crop as long as twelve or thirteen hours, and Colin found oats in the crop of a turkey eighteen and twenty hours after they had been swallowed. According to the latter author, a fowl weighing 500 or 600 grms. requires from four to six hours before 10 grms. of grains have passed out of the crop.

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[1] Designated often 'ventriculus succenturiatus.'

[2] Gegenbaur, *Comparative Anatomy*, pp. 557 and 558.

In the absence of specific information on this point, it is impossible to state definitely whether the liquid secreted by the crop subserves other than purely mechanical functions. In any case, it cannot be doubted that the latter are of the greatest importance. The fact that grains of wheat, oats, or barley, which have sojourned in the crop are not usually softened, renders it improbable that they there undergo any great chemical change. On the other hand, there can be no doubt that, as was appreciated by Spallanzani, the crop acts as a kind of 'hopper,' only allowing grain to pass into the *proventriculus* little by little, as the contents of the latter (grains and gastric juice) are admitted into the powerful corn-mill—the gizzard—where they are pounded together and converted into a liquid paste which is passed on to the small intestines.

Before passing from this subject, attention must be drawn to the well-known fact, studied in a special manner by John Hunter[1] and Duvernoy[2], but deserving of a histological and chemical research conducted from the stand-point of our present knowledge, viz. that in the pigeon, and doubtless other birds, the crop undergoes a change both in the male and female bird, during the incubation of the young, and for some weeks afterwards. Its mucous membrane becomes very vascular and hypertrophied, presenting marked folds and wrinkles. A milky liquid is poured out from the open mouths of the glands and accumulates in the cavity of the crop. It is said that this milky liquid can be regurgitated, and serves as the sole food of the young pigeons during the first days of their life[1].

Function of the proventriculus and gizzard.

It is in the proventriculus that are situated the glands which secrete the gastric juice. This fluid was first collected by Spallanzani and Réaumur, and afterwards examined by Tiedemann and Gmelin. These observers found the reaction of the juice acid, discovered that it contained hydrochloric acid and that it possessed the property of curdling milk. It appears certain that it is only in the gizzard that actual action of the gastric juice on the food takes place, its sojourn in the proventriculus being very short and its mechanical state opposing itself to a proper digestive action.

The function of the muscular division of the stomach (the gizzard) has been sufficiently explained in the above remarks as well as in connection with the mechanical function of the crop.

SECT. 5. DIGESTION IN THE HERBIVORA.

The first thing which strikes us in comparing the organs of digestion of herbivorous as contrasted with carnivorous and omnivorous animals is, firstly, the much greater length of their alimentary canal; secondly, its enormous capacity.

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[1] John Hunter, 'On a secretion in the crop of breeding pigeons for the nourishment of their young,' *Observations on certain parts of the Animal Œconomy*, London, 2nd ed. 1792, pp. 235—238.

[2] Duvernoy, see his edition of Cuvier's *Leçons d'Anatomie Comparée*, 2me édit., Paris, 1836.

Firstly, as to length. Whilst the ratio of the length of the alimentary canal of carnivora is to that of their body approximately as 4 : 1, and of man as 6 : 1, in the sheep the ratio is as 28 : 1, and in the large ruminants as 20—15 : 1[1].

Secondly, as to capacity. All animals whose food contains a large proportion of cellulose possess an alimentary canal of remarkable capacity.

We may form some idea of this fact by ascertaining the weight of the intestinal contents. Thus in the rabbit immediately after it has been fed, the contents of the stomach may weigh as much as one-tenth of the total weight of the body. The subjoined table, which exhibits a few of the data ascertained by Colin on this subject, shows clearly, firstly, the immense capacity of the whole alimentary canal in some herbivora: secondly, the relatively small capacity of the stomach in Solidungula (the horse) as compared with Ruminants: thirdly, the remarkable capacity of the large intestine, both in Solidungula and Ruminants, and its much larger capacity in the former than the latter.

TABLE EXHIBITING THE RELATIVE WEIGHTS OF THE CONTENTS OF THE STOMACH AND INTESTINES IN THE HORSE, BULL, COW AND RAM (ABRIDGED FROM COLIN)[2].

	Weight of Contents of Stomach	Weight of Contents of Small Intestine	Weight of Contents of Cæcum	Weight of Contents of Colon	Total Weight of Contents of Intestines	Total Weight of Contents of Stomach and Intestines
	kilos	kilos	kilos	kilos	kilos	kilos
Horse	5·000	7·500	11·000	36·200	54·700	59·700
Bull	98·000	11·500	1·000	8·500	21·000	119·000
Cow	67·000	6·000	0·300	3·700	10·000	77·000
Ram	7·200				1·600	8·800

The great capacity of the alimentary canal of herbivores is related to the fact of their food containing a large proportion of undigestible cellulose, so that the bulk and weight of the food which the animal must daily consume in order to obtain from it the albuminous, starchy, and fatty constituents which are essential to its maintenance is very large; moreover very large quantities of saliva are secreted and added to the dry food which is swallowed. We may assume that a horse of average size and weight will require, in order to keep in a state of nutritive equilibrium, about 7·5 kilo. of hay and 2·27 kilo. of oats, or if fed on hay alone at least 10 kilo. (22 lbs.); according to Colin's calculations, the saliva secreted by a horse during the mastication of a meal of 4 kilo. of hay amounts to 16 kilo, so that the daily food would, on this supposition, require 40 kilo. of saliva; in other words, the total weight of dry food and saliva entering the stomach during twenty-four hours would be 50 kilo. But to this we must add a quantity of water drunk, which cannot be estimated at less than 10 litres, so that the total ingesta mixed with saliva entering the stomach of the horse may be estimated as weighing about 60 kilo. or 132 lbs. per diem. When we reflect, further, on the bulk occupied by a quantity of hay weighing 22 lbs., we shall have an adequate explanation of the capacity of the alimentary canal of the horse. Taking now the case of the ox, we may calculate that if fed upon hay alone it would consume, on an average, about 15 kilo. (33 lbs.), with which would be mixed 60 kilo (132 lbs.) of saliva and from 15 to 25 litres of water, so that from 90 to 100 kilo. of matter would enter the *rumen* per diem.

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[1] C. A. Ewald, *Die Lehre von der Verdauung, etc.* Berlin, 1872, p. 22.

[2] Colin, *Traité de Physiologie Comparée des Animaux.* Paris, 1886 (see p. 900).

'The stomach of the horse is remarkable for its small size both in relation to the rest of the alimentary canal and to the large amount of food which the animal consumes. Thus while the stomach of a large dog may have a capacity of 6 litres, that of a horse may not contain more than from 10 to 18 litres[1].' The comparative smallness of the stomach is connected with the fact that when the food consumed is hay or straw, its stay in the stomach is, *for the most part*, a short one. In the horse, as in other herbivorous animals, under normal conditions the stomach at the commencement of a meal usually contains considerable quantities of undigested residues of previous meals. The newly ingested food partly forces out of the stomach some of the residual food which it contained, and partly becomes mixed with another portion of the residue. From the very commencement of the meal, however, some of the contents of the stomach pass through the pylorus into the duodenum. The quantity escaping does not, however, equal that which enters, so that the stomach gradually becomes distended. When a certain point is reached, however, the quantity admitted into the stomach is balanced by the quantity leaving it, so that the volume of the organ remains the same as long as the animal continues to eat. The meal finished, the passage of the contents of the stomach into the duodenum becomes materially slower, and it is only after many hours that the stomach can empty itself. When the aliment consumed is composed of oats, the process of gastric digestion is one which lasts longer, and which more nearly approaches that which goes on in the stomach of man or the carnivora.

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[1] J. G. McKendrick, *A Text-book of Physiology*, 1889, see Vol. II. p. 99.

The rapid passage of the solid gastric contents of the horse into the intestine has been determined both by Tiedemann and Gmelin and by Colin; these observers having administered foreign bodies to horses found them (unless their size was too great) in the intestine an hour or an hour and a-half afterwards. It is by the short contact with the gastric juice that Colin explains the fact that pieces of meat, oysters, &c., administered to horses are usually found to pass undigested into the intestine.

Amongst the phenomena connected with the alimentary canal of the horse which deserve to be mentioned, is the remarkable rapidity with which water passes out of the stomach in this animal and is conveyed towards and into the immensely capacious cæcum, where the absorption of water, and probably of soluble products of digestion, appears to have its chief seat. By adding ferrocyanide of potassium to the water drunk by horses, Colin found it in ten minutes in the ileum, at a distance of 20 metres from the stomach, though a somewhat longer time was needed before it entered the cæcum. To the processes which have their seat in the cæcum of the solidungula we shall refer when discussing the changes which cellulose undergoes in the alimentary canal of the Herbivora generally.

The stomach of Ruminants.

'The stomach of ruminants, such as the ox, sheep, goat, deer, chamois, elk, camel, dromedary, giraffe, &c.……….consists of four sacs—(*a*) the paunch or *rumen*, (*b*) the *reticulum*, (*c*) the *psalterium* or *omasum*, and (*d*) the *abomasum* or true stomach. The paunch is a sac of enormous capacity, in the ox capable of containing 100 litres, and in the sheep 4 to 6 litres. The mucous membrane is covered with pointed papillæ from 3 to 9 mm. in length, and the epithelial layer is of the stratified squamous variety. The paunch communicates with the lower end of the œsophagus and also with the *reticulum*. The paunch and reticulum are divided by a constriction and strong band of fibres from the psalterium and abomasum…… The *reticulum* has a capacity in the ox of 2 litres, and in the sheep of 0·2 litre. It shews, as its name indicates, a honeycomblike appearance, or reticulum, each cell of which is polyhedral. The height of the walls of these cavities may be from 10 to 15 mm. Its muscular coat is stronger than that of the paunch, and consists mainly of striated muscle, the fibres of which are continuous with those of the œsophagus. The walls of the reticular spaces also contain muscular fibres. The wall of the reticulum can thus contract more powerfully and speedily than that of the paunch. Fine papillæ abound, and the whole mucous membrane is covered with stratified squamous epithelium. The reticulum has three openings—a large one towards the paunch, a narrower one towards the psalterium, and a third which communicates with the œsophagus. At the opening into the psalterium, we find a sphincter surrounding an aperture narrowed by rugæ or folds, or by papillæ, so that matters entering into the psalterium from the reticulum pass through a kind of 'perforated partition.' The *psalterium* or *omasum* has a thin wall, and has two openings, one into the reticulum, already alluded to, and the others in the true stomach or abomasum. The mucous membrane of this cavity is thrown into the form of a number of leaves, projecting far into the lumen of the sac, and these are covered with small button-shaped papillæ, and by a layer of stratified pavement epithelium. This sac has in its walls involuntary muscular fibres forming strong longitudinal and circular layers. A layer of fibres also exists in the centre of each leaf or fold, in addition to a *t. muscularis mucosæ.* The structure of the *abomasum* or true stomach is like that described as characterising the stomach of man or the dog; it contains glands of the fundus and glands of the pylorus, and the mucous membrane is covered by cylindrical epithelium.

'The lower end of the œsophagus opens into the paunch, but at the left side a deep groove runs along the inner wall of the reticulum, from the entrance of the œsophagus into the paunch to the entrance of the reticulum into the psalterium. This may be called an œsophageal canal or groove. To understand its true relations we may regard the paunch and the reticulum as diverticula, or sacs developed from the *lower* wall of the œsophagus, and the psalterium as a diverticulum from the *upper* wall, supposing the animal to be on all fours in its normal position. We are now in a position to understand *rumination*[1].'

The process of 'rumination.'

The solid matters of the food, which have been subjected to the first or preliminary mastication, together with nearly the whole of the liquids (saliva and water), pass, after deglutition, into the *reticulum,* which is the real centre of the group of cavities which constitute the gastric apparatus of the ruminant. In virtue of its central position and of its powerfully muscular walls, the reticulum can divert the matter which flows into it either towards the right, into the rumen, of which it forms a preliminary chamber, or towards the left, into the *psalterium* and *true stomach.* The reticulum, in fact, in so far as the products of the first mastication are concerned, serves as a distributor of its products; in its cavity it stores only a certain part of the fluid which has reached it.

During rumination, the solid matters which have been stored in the rumen are, by the contractions of the rumen and through the instrumentality of the reticulum, regurgitated along the œsophagus into the mouth, in order to be subjected to a second and more perfect process of mastication ('chewing the cud'). When again swallowed, the finely comminuted alimentary particles, mixed with the saliva, pass along the œsophageal gutter directly into the psalterium. In this division of the stomach the comminution of the digested food is completed and it acts as a feeder and a regulator of the supply of matters in respect to the fourth or true stomach in which the real process of peptic digestion has its seat.

The chemical processes which have their seat in the stomach of Ruminants.

The mucous membrane of the rumen, as could be deduced from its structure (for it possesses no glands and is lined by stratified squamous epithelium), forms no secretion. In the rumen, the partially masticated vegetable matter is subjected, under the most favourable conditions of temperature, to the prolonged action of the saliva and, under the influence of its diastatic ferment, the conversion of starch into dextrins and sugars proceeds. At the same time much of the soluble matter of the food (salts, sugars, gums, &c.) must be extracted and pass into solution.

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[1] J. G. McKendrick, *A Text-book of Physiology,* Vol. II. pp. 100, 101.

Though the rumen produces no enzyme, and merely affords a suitable cavity in which the diastatic enzyme of the saliva may exert its action, it seems invariably to be the habitation of innumerable micro-organisms which provoke in its contents fermentations accompanied by the evolution of gases and by the development of fatty acids. In the rumen, a decomposition of cellulose due to the action of micro-organisms doubtless occurs.

The reticulum, it may be assumed, exerts no chemical action on the constituents of food, its function being, as we have stated, that of a distributor of matter. Nothing is known definitely as to the function of the *psalterium*. Tiedemann and Gmelin found its juice acid and discovered in it acetic acid, doubtless the product of fermentation. The function of the fourth, or true, stomach is exactly similar to that of the stomach of man or the carnivora. It would appear, however, from the observations of Bidder and Schmidt, that the proteolytic activity of the gastric juice which it forms is relatively feeble.

On the digestion of cellulose by the Herbivora.

Although the excrements of the herbivora contain large quantities of cellulose, the attention of physiological chemists has long been directed to the question whether any part of the cellulose introduced into the alimentary canal undergoes decomposition and, if so, whether the products are of such a nature as to be of some importance in nutrition. No enzyme is found in the alimentary canal nor is any unorganised ferment known which possesses the power of digesting cellulose. We are, however, in possession of facts which leave no room for doubt that cellulose can be decomposed through the agency of micro-organisms, and that this process occurs in the alimentary canal of the herbivora.

Tappeiner[1] found that cotton-wool when added to a solution of extract of meat mixed with bacteria obtained from the rumen underwent a process of fermentation accompanied by the evolution of gases and disappeared, fatty acids being found in the solution. v. Kniriem[2] shewed that during the process of digestion in herbivora an appreciable quantity of cellulose disappears, and even found that the cellulose of paper disappeared in its passage through the alimentary canal. Victor Hofmeister[3], by digesting hay made from young grasses with the liquid found in the intestines of slaughtered horses, observed the solution of a large proportion of the cellulose which it contained. In the process, which occurred rapidly, gases were evolved and the fermenting liquid became acid; no sugar was formed.

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[1] Tappeiner, 'Untersuchungen über die Gährung der Cellulose, insbesondere über deren Lösung im Darmkanale,' *Zeitsch. f. Biol.* Vol. xx. (1884), p. 52, and Vol. xxii. 1886, p. 105.

[2] von Kniriem, 'Ueber die Verwerthung der Cellulose im thierischen Organismus,' *Zeitsch. f. Biol.*, Vol. xxi. (1885), pp. 67—139.

[3] V. Hofmeister, 'Ueber Cellulose-Verdauung beim Pferde,' *Archiv f. wissensch. u. prakt. Thierheilkunde*, Vol. xi. (1885), Heft 1 and 2, quoted by Neumeister, *Phys. Chem.*, p. 235.

Henneberg and Stohmann[1], on the basis of these experiments, argued in favour of the actual nutritive value of cellulose, but their views have been controverted by H. Weiske[2] and others.

According to v. Kniriem, cellulose is essential to the herbivora for physical and not chemical purposes. He found that rabbits fed upon diet otherwise perfectly adequate to support life, but containing no cellulose, invariably died of intestinal inflammation, the cæcum being found to be filled with coherent pitch-like contents. When, however, horn shavings were mixed with food of the same composition, rabbits fed upon it throve, and the intestinal contents presented a normal appearance.

Whether cellulose plays any part or not as an active nutritive agent, we must, from all the researches hitherto made, arrive at the conclusion that in herbivora a considerable decomposition of this proximate principle occurs, brought about by the agency of the micro-organisms of the alimentary canal. Whilst this decomposition in Ruminantia probably has its chief seat in the *rumen*, in the Rodentia and Solidungula it is probable that it is chiefly localised in the capacious *cæcum*.

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[1] Henneberg and Stohmann, 'Ueber die Bedeutung der Cellulose-Gährung für die Ernährung der Thiere,' *Zeitsch. f. Biol.* Vol. xxi. (1885), p. 613.

[2] H. Weiske, 'Kommt der Cellulose eiweissersparende Wirkung bei der Ernährung der Herbivora zu?' *Zeitsch. f. Biol.*, Vol. xxii. (1886), p. 373.

[3] v. Kniriem, *op. cit.*

APPENDIX.

I.

NEUMEISTER'S VIEWS CONCERNING THE ALBUMOSES, AND THEIR RELATIONS.

(*Supplementary to pages* 131 *and* 132.)

AT page 131 of this book, a short space is devoted to an exposition of R. Neumeister's views concerning the albumoses. Quite apart from a mistake in print[1] (*hemi*-deutero-albumose appearing instead of *anti*-deutero-albumose), the account there given conveys so meagre and unsatisfactory an idea of the speculations of Neumeister, which are based on painstaking and valuable researches[2,3] that the Author here takes the opportunity of amplifying and amending it.

Neumeister adopts the hypothesis which Kühne advanced to explain the facts discovered by Schützenberger (p. 116), and especially by himself, viz. (refer to p. 119), that the albuminous molecule when subjected to hydrolytic agencies splits up into simpler bodies which belong to two distinct molecular groups—a *hemi*-group and an *anti*-group—which we may conceive actually to form part of the original albuminous molecule.

Neumeister, however, on the basis of actual experiment, has endeavoured, firstly, to establish the successive stages in the degradation of the albuminous molecule: secondly, to trace the relative distribution of the hemi- and anti-groups in the several products of decomposition: thirdly, to give a comprehensive schematic view of the manner in which the decomposition may be conceived to proceed. The reader must *in limine* be warned that such schematic views as those of Neumeister are neither advanced as ultimate nor do they lay claim to absolute truth. They are intended to aid us in our striving to discover and arrange the facts which shall ultimately lead us to formulate a correct theory, and may be likened to the poles and planks of the scaffold which enable us to construct a beautiful edifice, though they themselves will ultimately be taken down and no longer be seen.

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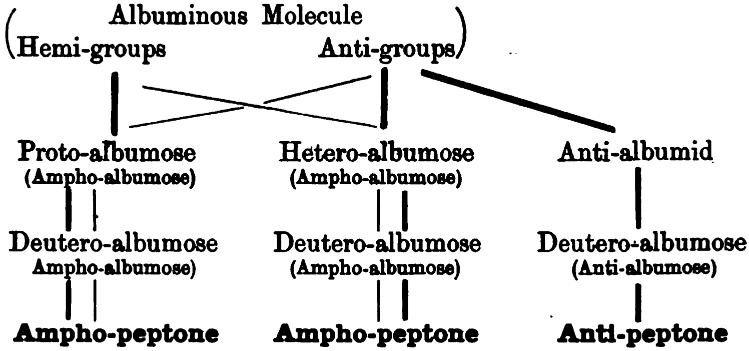
[1] Six lines from the bottom of page 131.

[2] Richard Neumeister, 'Zur Kenntniss der Albumosen,' *Zeitschrift f. Biologie*, Vol. xxiii. (1887), pp. 381—401; 'Bemerkungen zur Chemie der Albumosen und Peptone,' *Ibid.* Vol. xxiv. (1888), p. 267; 'Ueber die Reaktionen der Albumosen und Peptone,' *Ibid.* Vol. xxvi. (1890), pp. 324—348.

[3] The subjoined papers are not *so directly* connected with the precise subject under discussion as the three quoted above, but yet must be read by all interested in it, and they complete (so far as is known to the Author) the list of Neumeister's scientific contributions to the chemistry of the albumoses and peptones, if we except the account which he has given in his talented *Lehrbuch der physiologischen Chemie*, Erster Theil, Jena, 1893: 'Ueber Vitellosen,' *Zeitschrift f. Biologie*, Vol. xxiii. (1887), pp. 402—411; 'Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Eiweisskörper und Albumosen,' *Ibid.* Vol. xxvi. (1890), pp. 57—83; 'Zur Physiologie der Eiweissresorption und zur Lehre von den Peptonen,' *Ibid.* Vol. xxvii. (1891), pp. 309—374.

We commence our amended exposition of Neumeister's views by placing before the eyes of the reader the schema which represents them.



As was stated in the body of this book, Neumeister recognizes two so-called '*primary albumoses*': to wit, *proto*-albumose and *hetero*-albumose, into the formation of which, as the schema indicates, there enter both *hemi*- and *anti*-groups, resulting from the splitting up of the original albuminous molecule. The relative share which each of the groups takes is attempted to be indicated by the dark and light lines in the schema. Thus, according to Neumeister, *proto*-albumose is mainly built up of *hemi*-groups, though (as indicated by the finer diagonal line which joins it), in a minor degree, the molecular groups of the *anti*-moiety take a part in its construction. Conversely *hetero*-albumose is mainly derived from the *anti*-groups, but yet contain *hemi*-molecules.

From each of these primary albumoses, through the progressive activity of hydrolytic agencies, there may be derived '*secondary albumoses*' which, although not identical, yet are included under the generic term of *deutero-albumoses*. Both the primary albumoses, as well as the two deutero-albumoses derived from them, may be designated '*ampho*-albumoses' to indicate that they contain both *hemi*- and *anti*-molecular groups, though, as the schema indicates, in different relative proportions.

From these two deutero-albumoses, *which are derived from the primary albumoses*, by the further action of hydrolytic agents, we obtain ampho-peptones, but, according to Neumeister, *proto*-albumose yields an ampho-peptone in which the *hemi*-groups are chiefly represented, whilst *hetero*-albumose yields an ampho-peptone in which the *anti*-groups proponderate.

We must now approach the explanation of facts which are indicated in the right-hand column of Neumeister's schema. It has already been pointed out that under the action of certain agents, as for example when the albuminous bodies are subjected to long-continued boiling with acids[1], there is formed a body which Schützenberger called '*hemi-protein*' and Kühne '*anti-albumid*': Kühne, as has been stated, shewed that when subjected to long-continued digestion with trypsin, anti-albumid yielded, as a product of decomposition, a peptone (*anti-peptone*), but no trace of amido-acids. These facts are lucidly explained by Neumeister's scheme. We have to assume that under certain conditions (typically under the influence of acids) there are split off from the original albuminous molecule, groups of *anti*-molecules which constitute anti-albumid. The latter body, then, by the continued action of hydrolytic agents, as of trypsin, yields a deutero-albumose which is not, however, like those we have been considering, an *ampho*-albumose, for it contains no hemi-groups, and when finally digested will yield an *anti-peptone*, not an *ampho-peptone*, *i.e.* a peptone which when digested with trypsin will not furnish those products of decomposition (amido-acids) which are characteristically the products of the splitting-up of the bodies which contain *hemi*-molecules. We thus see that according to Neumeister's scheme, whilst we have one proto-albumose and one hetero-albumose, we may have three deutero-albumoses, differing in their constitution, as made out by a study of their products of decomposition.

It will be observed that Neumeister's scheme admits of a great many possibilities. We may conceive, for instance, of an almost endless variation in the constitution of both the primary and secondary albumoses, according to the method in which the decomposition of the albuminous molecule has been effected, as, for example, according to the extent to which the anti-groups of molecules have been split off to form anti-albumid.

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[1] See the account of Schützenberger's researches at p. 116, and Kühne's scheme of proteid decomposition by acids, p. 121.

II.

ON THE SEPARATION OF PROTO- FROM DEUTERO-ALBUMOSE.

(*Supplementary to pages* 125 *and* 129.)

The methods which have been described at pages 125 to page 129 for separating deutero-albumose have been shewn by Neumeister to be incapable of yielding the substance in a pure condition, as shewn by the fact that the product obtained always gives a precipitate, or at least a turbidity, when treated with a 2 per cent. solution of copper sulphate, whereas a solution of pure deutero-albumose is not precipitated by this reagent.

When a mixture of primary (proto- and hetero-) albumoses and of deutero-albumose is saturated with sodium chloride, the precipitate is composed of the primary albumoses. The precipitation of proto-albumose is not however a complete one, so that if acetic acid (or better still acetic acid saturated with sodium chloride) be added to the filtrate, the body which is thrown down is a mixture of proto- and deutero-albumose. When acetic acid saturated with NaCl no longer occasions a precipitate, the filtrate still contains deutero-albumose, which is now, however, entirely free from proto-albumose. In order to obtain the pure body, the solution, which contains free acetic acid as well as sodium chloride, is freed from these bodies by long-continued dialysis. On concentrating the aqueous solution and then adding alcohol, deutero-albumose is precipitated; the precipitate may then be dried by further treatment with absolute alcohol, the alcohol being allowed to evaporate spontaneously[1].

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[1] Refer to the following papers: R. Neumeister, 1. 'Zur Kenntniss d. Albumosen,' *Zeitsch. f. Biologie*, Vol. XXIII. (1887), pp. 381—401; 2. 'Ueber die Reaktionen der Albumosen und Peptone,' *Ibid.* Vol. XXVI. (1890), pp. 324—348.

III.

ON THE DIFFUSIBILITY OF ALBUMOSES AND PEPTONES.

(*Supplementary to pages* 135 *and* 141.)

The experiments of Funke[1] led him to assert that peptones in aqueous solution diffuse through animal membranes with much greater facility than other proteids, and Wittich, as well as Maly, Herth and others (refer to p. 141), repeating Funke's observations, but employing parchment paper instead of animal membranes, arrived at a very different conclusion. The matter has recently again been investigated (though as yet not in a complete manner) by Kühne[2]. Under the name of peptones, Funke and succeeding observers had worked with indefinite mixtures of albumoses and amphopeptone. Kühne, on the other hand, has experimented with solutions of the separate albumoses and with ampho- and anti-peptones, though these peptones were not quite free from albumoses. He has shewn that hetero-albumose is practically indiffusible, but that proto- and deutero-albumose possess tolerably high diffusive powers. Curiously, deutero-albumose, which according to Neumeister is a secondary albumose, appears to be less diffusible than proto-albumose.

The diffusibility of ampho-peptone and of anti-peptone is very much greater than that of either of the diffusible albumoses. Kühne has estimated that the diffusibility of both peptones (which strangely appear to possess the same diffusive power) is about one-fourth that of grape-sugar.

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[1] O. Funke, 'Das endosmotische Verhalten der Peptone,' Virchow's *Archiv*, Vol. XIII. (1858), p. 449.

[2] W. Kühne, 'Erfahrungen über Albumosen und Peptone. II. Zur Diffusion der Albumosen und Peptone,' *Zeitsch. f. Biol.* Vol. 29 (1893), p. 20.

IV.

KÜHNE'S NEW METHOD OF SEPARATING ALBUMOSES FROM PEPTONES AND FOR THE PREPARATION OF THE LATTER.

(*Supplementary to pages* 136 *and* 137.)

Since the sheet of this book in which the peptones were treated of passed through the press, a very important paper by Kühne has appeared[1], which is of so much importance in connection with the mode of separation of the albumoses and peptones that the leading facts which it contains must be referred to.

All the other methods of preparing the peptones which result from the action of pepsin and hydrochloric acid on the albuminous substances (ampho-peptones), such as the methods of Henninger and of Maly and Herth (described at page 136), yield, as products, mixtures of albumoses with peptones in which the former preponderate. It may indeed be positively asserted that until the appearance of the paper under discussion no ampho-peptones had been obtained which were free from albumoses.

It is one of the fundamental and most important facts connected with digestion by pepsin that, however active the solution of the enzyme, however careful the experimenter may be in keeping up the percentage of acid in the digestive mixture to the strength at which proteolysis proceeds most rapidly under the most favourable conditions of temperature (40°—45° C.), however long the process may go on (*i.e.* even though carried on for weeks and for months) large quantities of albumoses are found mixed with peptones.

After the publication of the paper of Wenz[2], it was, at first, believed that by simply saturating, in the cold, a mixed solution of albumoses and peptones with ammonium sulphate, it was possible to remove the whole of the albumoses from the solution, and Kühne and Chittenden's researches on peptones were all made on bodies purified in this manner[3]. Experiment however afterwards shewed that, in order to effect anything like an apparently complete separation of the albumoses from peptones, the mixture containing these substances should be saturated, whilst boiling, with ammonium sulphate. In whatever manner it may be carried out, a single saturation with ammonium sulphate is not, however, sufficient to remove all the albumoses. The difficulty, nay, the impossibility, of thus completely separating all traces of these bodies from peptones was pointed out by Neumeister[1].

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[1] W. Kühne, 'Erfahrungen über Albumosen und Peptone, I. Reinigung der Peptone von Albumosen,' *Separatabdruck aus der Zeitschrift f. Biologie*, 1893.

[2] Wenz, *Zeitschrift f. Biologie*, Vol. xxii. (1886), see p. 10. Refer to pages 124 and 134 of the present volume.

[3] Refer to page 137.

The principle of Kühne's new method.

"The sufficiently diluted mixture, containing albumoses and peptones having been freed from coagulable albumin and from albuminates in solution, is neutralised and saturated, at the boiling temperature, with ammonium sulphate. It is then allowed to cool and, thereafter, separated from the crystallised salt and mixture of albumoses which have separated. The filtrate is again heated and, as ebullition is commencing, it is rendered strongly alkaline by the addition of ammonia and ammonium carbonate; it is then again saturated with ammonium sulphate and allowed to cool. When cold, the mixture is filtered from the second precipitate of albumoses and from the ammonium sulphate which has separated. The filtrate is again heated (boiled) until all smell of ammonia has disappeared, again saturated when boiling with ammonium sulphate and then acetic acid is added, until the reaction is distinctly acid, an operation which leads to a third precipitate, which for the most part separates as the liquid cools. The quantities of the albumoses which necessarily separate are, as would be imagined, very different, according to the nature of the albuminous substance employed; they are, as a rule, greatest after long-continued digestions (*i.e.* in the latter, the amount of the second and third precipitations is larger, as compared with the first, than in digestions which have extended over shorter periods): it is greater in digestion with pepsin than in digestion with trypsin, and it varies with the nature of the albuminous substances which are acted upon. Thus the precipitate of albumoses which separates when the product obtained by digesting white of egg with trypsin is subjected to the third precipitation previously referred to (viz. when acetic acid is added to the hot solution saturated with ammonium sulphate) is surprisingly abundant[2]."

Method of obtaining large quantities of amphopeptone (Kühne).

In order to obtain large quantities of peptones free from albumoses, Kühne recommends the use of Witte's so-called 'peptonum siccum' (refer to page 129), a substance which is prepared by digesting fibrin with pepsin and hydrochloric acid, and which contains the three chief albumoses (proto-, deutero-, and hetero-albumose) in remarkably fluctuating proportions[3]. This preparation must be subjected to renewed digestion for a period of many weeks (six or eight) with a highly acid solution of pepsin. The acid pepsin solution employed should, in the first instance, contain 0·5 per cent. of HCl, and, after the albumoses have been introduced and digestion has commenced, hydrochloric acid must be added, in the proportion of at least 3—4 per cent. of the weight of the albumoses subjected to digestion. Kühne states that if these conditions be fulfilled the albumoses digested may amount to 4 or 5 per cent. of the acid pepsin mixture. The Author having three times repeated Kühne's method, on a large scale, with digestive mixtures containing varying quantities of albumoses, is inclined to think that a greater proportionate yield of amphopeptone is obtained when the percentage of albumoses is low than when it is high. It must be stated, however, that the difficulties, the labour, and the expense of the operations increase enormously with the increase of dilution.

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[1] R. Neumeister, 'Bemerkungen zur Chemie der Albumosen und Peptone,' *Zeitsch. f. Biologie*, Vol. xxiv. (1888), p. 267.

[2] Freely translated from Kühne, *op. cit.* pp. 2, 3.

[3] The experience of the Author, in reference to Witte's 'peptone,' agrees on this point with that of Neumeister. Two samples which he investigated contained deutero-

To prepare the pepsin solution which should be employed in making large quantities of amphopeptone, the following process should be employed (which is the same as that described at page 83, the final dialysis being omitted):—the mucous membrane of the cardiac case of the stomach of two or three pigs, having been washed and minced, is digested for at least a week with dilute hydrochloric acid (containing 0·5 per cent. HCl). The dark-coloured artificial gastric juice thus obtained is, after filtration, saturated with ammonium sulphate, which precipitates the albumoses; these carry down with them the whole of the pepsin. The precipitate is washed with a saturated solution of ammonium sulphate; the latter is separated as completely as possible from the precipitate, which is ultimately pressed between filter paper. The pepsin-containing albumoses are found to be entirely soluble in 0·1—0·5 solution of HCl, furnishing a clear and colourless solution, which possesses intense peptic activity.

The successive processes of precipitation with ammonium sulphate which have been referred to as necessary to the complete separation of albumoses and which have all to be carried out at the boiling temperature, necessitate very large quantities of ammonium sulphate, so that in order to obtain one hundred grammes of amphopeptones free from albumoses the experimenter will employ, in the successive operations, many kilogrammes of the ammonium salt. The albumoses which separate when ammonium sulphate is added, to complete saturation, to the mixture of albumoses and peptones which has been rendered alkaline by ammonium carbonate and ammonia, at first partially separate in a flocculent form, but in great part as a dark brown melted scum, which is best removed by a perforated ladle; the brown colour is obviously the result of processes of oxidation brought about by the atmospheric oxygen in the presence of free ammonia. This dark scum rises to the surface again and again and must be removed.

albumose and proto-albumose (the former preponderating very largely) with only a trace of hetero-albumose and no dys-albumose.

Separation of peptones from ammonium sulphate.

When the successive saturations with ammonium sulphate, at a boiling temperature, have been effected, the experimenter is, at last, in possession of a large quantity (perhaps a great many litres) of a cold saturated solution of ammonium sulphate containing amphopeptones. As pure water dissolves, at ordinary temperatures, about 70 per cent. of its weight of ammonium sulphate, it follows that each kilo of the fluid contains about 700 grammes of ammonium sulphate.

In order to separate the larger part of this salt, the solution is briskly boiled, evaporation being aided by keeping it continually stirred (preferably by an automatic stirrer). When sufficiently concentrated, the whole is exposed to as low a temperature as possible, and the concentrated solution is separated, by decantation and thorough draining, from the crystalline mass which has formed.

The now yellow-coloured solution is mixed with one-fifth of its volume of alcohol, which causes an abundant separation of crystals of ammonium sulphate; after their separation, the turbid liquid is poured off, when it is observed to separate into two layers, a lighter supernatant layer, which contains much alcohol with a large quantity of peptone, and a heavier layer, which contains a large quantity of ammonium sulphate in solution, but relatively little peptone.

These two layers are separated from one another, as far as possible by simple decantation, and then by employing a separating funnel. The heavier solution, rich in ammonium sulphate, previously referred to, is treated with successive quantities of alcohol; each addition by the latter causes the separation of additional quantities of yellowish peptone and alcohol-holding supernatant liquid, which floats on the surface of the aqueous solution of ammonium sulphate. The latter on each addition of alcohol further furnishes deposits composed of crystals of ammonium sulphate.

The alcoholic solution, comparatively rich in peptones and poor in ammonium sulphate, which results from all these operations, having been placed in a stoppered bottle, is exposed to a very low temperature, for at least twenty-four hours. The yellow alcoholic solution may then be decanted from the thick and hard crystalline crust of ammonium sulphate which has separated.

It is now boiled, so as to drive off the whole of the alcohol, and afterwards boiled with barium carbonate. The latter process serves to decompose the ammonium sulphate yet present, barium sulphate being precipitated and ammonia evolved. Kühne recommends that ammonia and ammonium carbonate should be added during the process of decomposition with barium carbonate, and states that by proceeding in this way he has succeeded in obtaining a solution of peptone which contained neither sulphuric acid nor barium in solution. If this desirable result has been attained, the solution of peptones is filtered; it may then be further boiled until all ammonia is expelled and is concentrated to almost syrupy consistence on the water bath. The syrupy liquid is then poured into pure (absolute) alcohol, when the amphopeptone is at once precipitated, and soon assumes the appearance of white or greyish-white crusts. These may be further purified by solution in very small quantities of water and reprecipitation by absolute alcohol. They may then be dried in vacuo over sulphuric acid. If it be not an object to obtain the amphopeptone in as colourless a condition as possible, the peptone which has been precipitated by alcohol may be dried as follows. It is dissolved in water and the solution is boiled so as to drive off every trace of alcohol; it is then evaporated, in a porcelain capsule, to dryness on the water bath, and may be kept in well-stoppered bottles, or in sealed tubes. The so-called dry substance thus obtained, if however further heated to 110° C., continues to lose water for a long time and then the dark-coloured residue thus obtained possesses the intensely hygroscopic characters first observed by Kühne; when dropped into water it dissolves with the evolution of heat and with a hissing noise, in the same manner as phosphoric anhydride does under the same circumstances.

It sometimes occurs that after boiling with barium carbonate and filtering, the peptone solution is found to give a precipitate with dilute sulphuric acid, indicating the presence of barium peptone. In this case the whole solution must be *very cautiously* treated with just enough very dilute sulphuric acid as is needed exactly to precipitate all the barium present, and thereafter the processes previously described should be carried out.

The experimenter cannot, however, be sure that his long and tedious work has been successful, until having dissolved a sample of the dry peptone finally obtained he submits the solution to the three sets of precipitations which formed the essence of the whole operation. If ammonium sulphate produces any precipitate when added (to saturation) to a boiling solution in the neutral, the alkaline, and the acid, conditions, the whole of the product must be worked up *de novo*.

According to the Author's experience, however, if the whole of the operations which have been described are carried out with thoroughness and intelligence, neither time nor material being spared, the product obtained is found to be absolutely free from albumoses. He has, however, found that even when the solution of amphopeptone, filtered from the precipitate of barium sulphate, gives absolutely no precipitate with either barium chloride or with dilute sulphuric acid, a very concentrated solution of the amphopeptone ultimately obtained is found to contain a trace of barium. It would appear therefore that peptones in solution prevent the *perfect* precipitation of traces of barium by sulphuric acid.

V.

NOTES AND ADDITIONS ON THE NATURE OF THE ACIDS OF THE GASTRIC JUICE AND GASTRIC CONTENTS[1].

1. ON THE COLOUR REACTIONS WHICH MAY BE EMPLOYED IN THE INVESTIGATION OF THE ACIDS OF THE GASTRIC JUICE AND GASTRIC CONTENTS.

(*Supplementary to pages* 92—95 *and* 179.)

The reader who has perused the account given of the various colouring methods which may be employed either as indicators of the total acidity of the gastric juice, to discover the presence of free hydrochloric acid, or of free acids, mineral and organic, will be conscious of the want of more definite information as to the special advantages which certain reagents possess over others. This want is supplied by the results of the observations of Martius and Lüttke, and to certain of these we shall draw the attention of the reader.

Behaviour of the compounds of the albuminous substances with HCl towards litmus[2].

The compounds of albumin with hydrochloric acid behave *towards litmus* as free acids; it follows therefore that even in the absence of free HCl, litmus may be reddened by the contents of the stomach. It is indeed very rare that the contents of the stomach possess a neutral or alkaline reaction. Litmus is, unlike phenolphthaleïn, not affected by solutions of acid phosphates. Some hydrochlorates of organic bases give an alkaline reaction with litmus, whilst with phenolphthaleïn they exhibit an acid reaction[3].

Behaviour of the compounds of the albuminous substances with HCl towards 'phenolphthaleïn.'

Phenolphthaleïn is a body which has of recent years come into very general use as an indicator in the titration of acids and alkalies, and which possesses special advantages when it is desired to determine the total acidity of the gastric juice. It is therefore essential to know in what respect its action differs from that of litmus.

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[1] These notes and additions are mainly based on the researches and writings of Martius and Lüttke. See *Die Magensäure des Menschen*. Kritisch und experimentell bearbeitet von Dr F. Martius, A. O. Professor u. Direktor der med. Poliklinik in Rostock und J. Lüttke, Chemiker in Rostock. Stuttgart, Verlag von Ferdinand Enke, 1892.

[2] Martius and Lüttke, *op. cit.* 39.

[3] Salkowsky und Kumagawa, 'Ueber den Begriff der freien und gebundenen Salzsäure im Mageninhalt,' Virchow's *Archiv*, Vol. cxxii. (1890), pp. 235—252.

Phenolphthaleïn is a colourless crystalline powder, which is sparingly soluble in water but abundantly soluble in alcohol, the solution in the latter possessing a pale yellow colour. The addition of alkalies causes this yellow solution to assume an intense red colour, which is bleached by the addition of acids and by the action of CO_2.

Phenolphthaleïn is invariably added in alcoholic solution to the liquids of which the reaction is to be determined. As previously stated, acid phosphates behave towards it as acids, *i.e.* in their presence the addition of alkali must be continued until they are converted into perfectly neutral salts before the red colouration makes its appearance. Acid compounds of albuminous substances behave towards phenolphthaleïn as free acids.

Inasmuch as tincture of litmus cannot, with expediency, be added directly to the gastric contents and the use of litmus paper is inconvenient, phenolphthaleïn is to be preferred to litmus in determining the total acidity of the gastric juice.

To summarize:—*in litmus and phenolphthaleïn we possess indicators which merely inform us of the acid reaction of the gastric juice, but furnish us with no information as to whether a free acid be present or not.*

Congo-red and tropæolin.

As was previously said (p. 94), these two reagents possess special value in the investigation of the gastric juice and of the contents of the stomach, inasmuch as *they are not affected by salts with acid reactions or by compounds of albuminous substances with hydrochloric acid.*

According to Martius and Lüttke, however, *no reliance can be placed on these reagents as indicating whether the free acid of the gastric juice is organic or mineral.*

Paper stained with congo-red is to be employed as the readiest and most delicate test of the presence of free acids in the gastric juice, whilst in determining the total amount of *free acid* (as distinguished from the *total acidity*) by the aid of a standard alkaline solution, a solution of 00 tropæolin (1 part dissolved in 10 of weak spirit) should be employed.

Phloro-glucin-vanillin and 'resorcin,' only affected by mineral acids.

Whilst congo-red and tropæolin indicate the presence of free acids in the gastric juice, they cannot be relied upon to tell us whether the acid be organic or mineral. Günzburg's reagent[1] (Phloro-glucin-vanillin), described at p. 94, is totally unaffected by organic acids, but indicates the existence of free mineral acids. If, therefore, the contents of the stomach furnish a positive reaction with it, we can assert that they contain free hydrochloric acid. To the directions given at p. 94 for the employment of this reagent, should be added that a few drops of the gastric juice are sufficient to exhibit it, and that unless the quantity of acid present be extraordinarily small, the red colour of the dried stain is seen to be due to extremely slender microscopic crystals.

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[1] Günzburg, 'Neue Methode zum Nachweise freier Salzsäure im Mageninhalt,' *Centralblatt f. klin. Med.* 1887, No. 40.

A solution of Resorcin, employed in the same manner as Günzburg's reagent has been recommended by Boas[1], and can be employed in its stead. It is only influenced by free mineral acids and not by the compounds of hydrochloric acid with the albuminous substances or with organic bases. The solution employed is made by dissolving 3 grms. of resorcin and 3 grms. of cane-sugar in 100 grms. of spirits of wine.

2. ON THE SYSTEMATIC USE OF CERTAIN COLOUR REACTIONS IN DETERMINING THE PRESENCE OF MINERAL AND ORGANIC ACIDS IN THE STOMACH CONTENTS, AND ON THE QUANTITATIVE ESTIMATION OF THE 'TOTAL ACIDITY' AND OF THE 'ACIDITY DUE TO FREE ACIDS' (MARTIUS AND LÜTTKE)[2].

1. *Determination of Reaction. Is hydrochloric acid present?*

1. Litmus (blue). The contents of the stomach are tested with delicate blue litmus paper. It is, as has been said, only most rarely that the reaction is found to be neutral or alkaline.

If the reaction be acid, it may be due (*a*) to HCl only: (*b*) to organic acids: (*c*) to a mixture of HCl and organic acids: (*d*) to acid salts, or to a mixture of the latter with the acids previously referred to.

2. Congo-red. If paper stained with congo-red does *not* assume a blue colour, free acids are absent; it follows that an acid reaction previously observed with litmus must have been due to acid salts

If congo-red is turned blue, the stomach contents contain either free hydrochloric acid or free organic acids, or both.

To determine whether hydrochloric acid is present, the phloroglucin-vanillin test should be employed.

3. Phloro-glucin-vanillin. If this reagent furnishes a negative, whilst congo-red furnished a positive, reaction, the acid or acids present must be organic and no free hydrochloric acid be present.

If the reaction be positive, the presence of *free* hydrochloric acid is proved, whilst the simultaneous presence of organic acids is not excluded. A judgment on the latter point can only be arrived at, according to Martius and Lüttke, by determinations of (1) total acidity; (2) the acidity due to free acids; (3) the total quantity of chlorine present; (4) the quantity of chlorine present as chlorides.

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[1] Boas, 'Ein neues Reagens für den Nachweis freier Salzsäure im Mageninhalt,' *Centralbl. f. klin. Med.* 1888, No. 45.

[2] *Op. cit.* pp. 66, 67 and 115.

In the opinion of the Author, however, the method of the 'coefficient de partage,' &c. offers the most direct, as well as the simplest manner of settling the question.

2. *Quantitative determination of total acidity.*

In determining the acidity of the contents of the stomach it is absolutely essential, as Martius and Lüttke have conclusively proved, (1) to employ the unfiltered contents instead of, as has hitherto been the practice, the filtrate separated from the solid matters held in suspension, inasmuch as the degree of activity of the latter stands in no definite relation to the fluid which surrounds them.

20c.c of the thoroughly shaken contents of the stomach are measured by means of a graduated flask, and, after the addition of 3 to 4 drops of solution of phenolphthaleïn, are diluted, in another graduated flask, to the volume of 300 c.c. After thoroughly shaking, 150 c.c. of this mixture are poured into each of two beakers possessing a capacity of 200 c.c. and which are placed, side by side, on a sheet of white paper. Decinormal solution of sodium hydrate is then allowed to flow from a burette into one of these beakers, until the red reaction just appears. *The transition in tint can be readily observed, if both beakers are looked at, side by side.* Having made the first determination, it is repeated with the second portion.

In accordance with the very practical suggestion of Ewald, it is now the practice to express the value of the total acidity of gastric juice not in terms of the absolute amount of alkali required to neutralise 100 c.c. of gastric juice, but by the number of cubic centimetres of decinormal solution of sodium hydrate employed for the same purpose. Thus if we state that the acidity of the gastric contents equals 50, we signify that 100 c.c. would be neutralised by 50 c.c. of decinormal sodium hydrate.

3. *Quantitative determination of Free Acids.*

This determination is carried out as the determination of total acidity; solution of tropæolin (1 part to 10 of diluted spirit) is, however, substituted for phenophthaleïn. The transition from yellow to red is readily seen when the contents are suitably diluted and the quantity of the diluted fluid is not too great. About 50 c.c. should be employed[1].

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[1] Martius and Lüttke, *op. cit.* pp. 66 and 67.

3. ADDITIONAL METHODS OF DETERMINING THE ACIDS AND ESPECIALLY THE AMOUNT OF HCl IN THE GASTRIC JUICE.

(*Supplementary to pp.* 95—100 *and* 178—182.)

In addition to the methods, which have been described in the body of this work, for the determination of the hydrochloric acid of the gastric juice, a variety of new methods and of modifications of the older methods have within recent times been introduced. It would be impossible even to refer to all of them, and only those which possess special importance and which present peculiar features will be discussed.

1. *The Method of Cahn and v. Mering*[1] *for the determination of the total hydrochloric acid, the volatile acids and the lactic acid of the stomach contents.*

50 c.c. of the filtered stomach contents are distilled over a naked flame, until three-fourths of the liquid has distilled: the volume of the fluid in the retort is again made up to 50 c.c. and again three-fourths of the fluid distilled off. The distillate contains the volatile acids, the amount of which is determined by titration with decinormal soda. The residue in the retort or flask is shaken six successive times with 500 c.c. of ether, in order to extract the whole of the lactic acid. The united extracts are freed from ether by distillation and the lactic acid determined in the residue by titration. To the concentrated stomach contents, after treatment with ether, an excess of freshly precipitated cinchonin is added, until the reaction becomes neutral, and the mixture is then washed by means of chloroform into a separating funnel and shaken 4 or 5 times with fresh quantities of chloroform. The united chloroform solutions are freed from chloroform by distillation, the residue is dissolved in water, acidulated with nitric acid, and the chlorine present precipitated by adding an excess of silver nitrate. The chloride of silver is determined, *lege artis*, gravimetrically and the amount of hydrochloric acid corresponding to it calculated by multiplying its weight by 0·25427.

As will be obvious to the chemist, this method is costly, complex, and difficult, and presents inherent defects which must militate against its accuracy, the chief of which consists in the large quantities of ether which are employed in order to dissolve the lactic acid and which take up not inconsiderable quantities of the hydrochloric acid (Martius and Lüttke).

The process of Cahn and v. Mering, of which the most interesting feature was suggested by a striking experiment of Rabuteau (see foot-note, p. 93), has been simplified by McNaught[1], though according to Martius and Lüttke the modifications only increase the chances of error attaching to the original method.

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[1] Cahn and v. Mering, 'Die Säuren des gesunden und kranken Magens,' *Deutsch. Archiv f. klin. Med.* Vol. xxxix. (1886), p. 239.

2. *The Method of Sjöqvist[2] modified by v. Jaksch[3] of determining the total HCl in the stomach contents.*

This method rests upon the fact that when gastric juice or the contents of the stomach are evaporated to dryness with barium carbonate, the free acids combine with barium. On igniting the residue, the organic barium salts furnish insoluble barium carbonate, whilst the chloride of barium formed by the hydrochloric acid of the gastric juice is soluble in water, which may be used to extract it.

10 c.c. of unfiltered stomach contents are measured into a platinum or nickel capsule, and a drop of tincture of litmus is added. Absolutely pure barium carbonate (*i.e.* absolutely free from chlorine) is then added, until the mixture is no longer red, care being taken not to add an unnecessary excess of the barium salt. The capsule is now heated to dryness on the water bath, in an atmosphere free from HCl. The capsule is then ignited until all the organic matters are destroyed, and the residue is repeatedly extracted with boiling water and the solution filtered. The volume of the clear filtrate should not exceed 80—100 c.c. The quantity of barium which it contains should then be determined gravimetrically by precipitation with dilute sulphuric acid, &c. The quantity of barium sulphate multiplied by 0·3132 gives the amount of HCl present in 10 c.c. of the gastric juice. The control determinations made by v. Jaksch have shewn that, as carried out by him, the method is one of great exactitude. The method would appear to be theoretically a very perfect one if the barium determination be carried out gravimetrically by an experienced worker. Martius and Lüttke have, however, found that when the quantity of free HCl is small and the greater part of the acid is in combination with albuminous substances, the results obtained by Sjöqvist's method are frequently much below the truth. They believe that this may depend upon an imperfect decomposition of the acid albuminous compound and suggest that by diluting the mixture of gastric juice and barium carbonate and gently heating it the difficulty may be got over[4].

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[1] McNaught, *Medical Chronicle*, March, 1887. The Author has not had the opportunity to see the original paper.

[2] Sjöqvist, 'Eine neue Methode, freie Salzsäure im Mageninhalte quantitativ zu bestimmen,' *Zeitsch. f. phys. Chemie*, Vol. XIII. p. 1.

[3] v. Jaksch, 'Zur quantitativen Bestimmung der freien Salzsäure im Magensafte,' *Monatshefte f. Chem.* Vol. X. (1889), pp. 211—213.

[4] Martius and Lüttke, pp. 85 and 86.

3. *The Method of Hayem and Winter*[1] *for determining the free and combined HCl of the gastric contents.*

The reader is referred either to the original work of Messrs Hayem and Winter for a full description of their method, or to the exhaustive and critical examination of it contained in Martius and Lüttke's book[2]. As the latter authors have very candidly pointed out, the method of Hayem and Winter is based in part on a philosophical desire not only to determine the total quantity of hydrochloric acid in the gastric contents, and the total quantity existing in a free condition, but likewise the quantity which exists in organic combinations. Unfortunately, however, the means employed have not been adequate to the end in view and the method neither enables us to determine the proportion of the free nor of the combined hydrochloric acid.

Hayem and Winter measure out 5 c.c. of the filtered gastric contents into three crucibles, which we shall distinguish as *a*, *b* and *c*. To *a* is added an excess of sodium bicarbonate, so as to combine with the whole of the hydrochloric acid. All three crucibles are then placed on the water bath and their contents ultimately dried at a temperature of 100° C. The residue contained in crucible *a* is ignited for a few minutes over a naked flame and the carbonised mass is repeatedly extracted with distilled water and a little nitric acid. The solution is then neutralised with sodium carbonate and heated for some time to drive off the carbon dioxide.

The amount of chlorine which the solution contains is then determined by means of decinormal silver nitrate solution, potassium chromate being used as an indicator. From the amount of the decinormal silver solution employed, the total chlorine ('*chlore total*') is found.

The residue in crucible *b*, after being dried, is treated with concentrated solution of soda in excess and, after evaporation, the residue is ignited. The amount of chlorine which it contains is then determined as in the case of the residue in crucible *a*. By deducting the amount of chlorine found in *b* from that found in *a*, Hayem and Winter calculate the amount of free chlorine ('*HCl libre*').

The residue in crucible *c* is simply ignited and the chlorine in the residue determined in the same manner as in the case of *a* and of *b*. The quantity of chlorine found in *c* corresponds to the chlorides and is designated '*Chlore fixe*.'

The radical fallacy of Hayem and Winter lies in the assumption that when the gastric contents are evaporated to dryness, precisely the same quantity of hydrochloric acid will be volatilised as existed in the free condition in the juice, a supposition which is not merely theoretically improbable but, as the researches of Martius and Lüttke have shewn, is absolutely untrue. The total quantity of HCl can be legitimately deduced, as H. and W. suppose, from the chlorine determinations in a and c $(a-c)$. On the other hand, the value (H) of the free hydrochloric acid cannot be deduced as they pretend $(H=a-b)$, and the same remark applies to the value of the combined hydrochloric acid (c) which they state as $=b-c$.

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[1] Hayem et Winter, *Du Chimisme stomacal*, Paris, 1891.

[2] Martius and Lüttke, *op. cit.* pp. 94—101.

4. *Lüttke's method*[1] *of determining the total quantity of Hydrochloric Acid in the Gastric Contents.*

(A) *The principles on which the method is based.*

The contents of the stomach in the state of health contain, in addition to hydrochloric acid, certain chlorides of the alkalies and alkaline earths, to wit, sodium, potassium, calcium and magnesium chlorides. Ammonium chloride, on the other hand, has hitherto only been discovered in the contents of the stomach in uræmia.

The hydrochloric acid of the gastric contents is partly in the free condition and partly combined with albuminous substances.

Lüttke's process depends upon the theory, which is based on a large number of experimental facts collected by Martius and himself, that when the gastric juice or gastric contents are ignited (at a temperature much below that at which the chlorides would volatilise) the entire quantity of hydrochloric acid is evolved, *i.e.* not only that which exists in the perfectly free condition, but also that which is in combination with albuminous bodies, whilst the chlorine which is in combination with bases remains in the ignited residue. By determining, therefore, the total quantity of chlorine contained in the stomach contents and then that of the chlorine in the ignited residue, and by subtracting the latter from the former, there is obtained the amount of chlorine which corresponds to the total hydrochloric acid of the gastric contents. It is obvious that the practicability of a method based upon these considerations (assuming the original supposition to be correct, viz. that the whole of the hydrochloric acid existing free *and* in organic combination is evolved) will depend upon a method being available for the very accurate estimation of the total chlorine present in a complex organic mixture (such as the contents of the stomach), without having to subject this to any process for destroying the organic substances which it contains. Such a process Lüttke has found in Volhard's remarkably fine method for the estimation of chlorine, which has already supplanted all others in the determination of the chlorine contained in the urine[2, 3, 4, 5].

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[1] J. Lüttke, 'Eine neue Methode zur quantitativen Bestimmung der Salzsäure im Mageninhalt,' *Deutsche med. Wochenschr.* 1891, p. 1325, and more fully developed in Martius and Lüttke's *Die Magensäure des Menschen*, see pp. 101—114.

[2] Volhard, *Ann. d. Chemie u. Pharm.*, Vol. cxc. p. 1.

[3] F. Falk, *Ber. d. deutsch. chem. Gesellsch.*, Vol. VIII. p. 12.

[4] C. Arnold, 'Kurze Methode zur massanalytischen Bestimmung der Chloride im Harn,' *Zeit. f. phys. Chemie*, Vol. v. (1881), p. 81.

[5] E. Salkowski, 'Ueber die Bestimmung der Chloride im Harn,' *Zeitsch. f. phys.*

The principle of Volhard's process for determining Cl.

Volhard's method of determining chlorine as applied to the present object depends upon the fact, firstly, that in the presence of *strong* nitric acid, silver nitrate completely precipitates the chlorine and sulphocyanogen which may be present in a solution.

Secondly, that the precipitates which silver nitrate gives with albuminous bodies, with some organic acids, &c. are insoluble in strong nitric acid. If then to an organic mixture, such as the gastric contents or pure gastric juice, we add a known quantity of a standard solution containing silver nitrate in presence of a large quantity of strong nitric acid, taking care that the silver added is more than sufficient to precipitate all the chlorine, if we filter the mixture and determine in the filtrate the quantity of silver remaining (*i.e.* which has not combined with the chlorine), we shall at once know how much chlorine the mixture contained.

Thirdly, upon the fact that when a solution of ammonium sulphocyanate is added to a strongly acid solution of silver, *containing some ferrous sulphate*, a curdy precipitate of silver sulphocyanate falls, the reaction being shewn in equation 1:—

$$(1) \quad AgNO_3 + NH_4CNS = AgCNS + NH_4NO_3.$$

This precipitate at once redissolves with the production of a blood-red colour, due to the formation of sulphocyanate of iron, as shewn by equation 2:—

$$(2) \quad Fe_2(SO_4)_3 + 6NH_4CNS = Fe_2(CNS)_6 + 3NH_4SO_4.$$

But no sooner has the red colour been observed than it disappears, *so long as any silver remains in solution*, the iron sulphocyanate taking part in the reaction which is shewn in equation 3:—

$$(3) \quad Fe_2(CNS)_6 + 6AgNO_3 = Fe_2(NO_3)_6 + 6AgCNS.$$

It is only when the whole of the silver has been precipitated as silver sulphocyanide, that the blood-red colouration due to ferric sulphocyanide persists; the persistence of the red colouration indicates, therefore, the termination of the process, and, if the strength of the solution of ammonium sulphocyanide be known, the quantity of silver in the solution can be at once calculated.

(B) *The standard solutions needed in Lüttke's process.*

1. Deci-normal solution of silver nitrate.

16·997 grms. of dry and pure $AgNO_3$ are dissolved in about 900 c.c. of dilute nitric acid containing 25 per cent. of HNO_3, 50 c.c. of the 'liquor ferri sulfurici oxy-

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Chemie, Vol. v. (1881), p. 285; Salkowski u. Leube, *Die Lehre vom Harne*, Berlin, Hirschwald, 1882, see p. 168.

dati' of the German Pharmacopœia is added[1]; the mixture is then diluted with distilled water to the volume of 1000 c.c. Instead of taking exactly one-tenth of an equivalent of $AgNO_3$ in grammes, somewhat more, say 17·5 grms., may be taken, and the exact *titre* determined, *lege artis*, by means of a perfectly exact decinormal solution of hydrochloric acid. Except in the case of a trained chemist, the simpler plan will offer least chances of error.

2. Decinormal solution of ammonium sulphocyanate.

This solution should contain 7·6 grms. of pure NH_4CNS per litre. In order to prepare it, 8 grms. of the pure salt (as sold) are dissolved in 1000 c.c. of water. This solution must next be standardised against the standard acid solution of $AgNO_3$.

With this object, 10 c.c. of the silver solution are measured out into a beaker, and 150 to 200 c.c. of water are added. The sulphocyanate solution is then allowed to flow from a burette into the diluted silver solution, until the first appearance of a permanent reddish colouration. Supposing 9·7 c.c. were required, then 970 c.c. of the sulphocyanate solution would have to be diluted to 1000 c.c.

Finally, the diluted solution is tested against the accurately prepared decinormal silver solution, so as to ascertain that they absolutely correspond.

(C) *The Actual Process of Analysis.*

Lüttke's process includes two determinations: firstly, that of the total quantity of chlorine contained in the contents of the stomach; this quantity we shall designate a: secondly, that of the chlorides remaining in the incinerated residue of the contents; this quantity we shall designate b. Having made these determinations, the quantity of the total hydrochloric acid, *both free and in organic combination*, will be deduced from the value of $a - b$. The stomach contents are measured out in small graduated flasks of 10 c.c. capacity, and, for reasons already adduced, the solid matters are not separated by filtration from the liquid in which they are suspended.

a. Determination of the total chlorine.

10 c.c. of the thoroughly mixed (shaken) gastric contents are poured into a graduated flask of 100 c.c. capacity. 20 c.c. of the decinormal acid silver solution are added, the whole is shaken and set aside for 10 minutes.

In the event of the stomach contents being strongly coloured, they may be decolourised by the addition of 5 to 10 drops of a solution containing one part of potassium permanganate dissolved in 15 parts of water.

This addition (which is rarely necessary) *must only be made after all the chlorine has combined with silver, otherwise the permanganate*

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[1] The 'Liquor ferri persulphatis' of the British Pharmacopœia may be substituted for the German preparation. The former is a more concentrated solution of ferric sulphate, having a specific gravity of 1·441, whilst the latter varies between 1·317 and 1·319.

would decompose the HCl, liberating chlorine; and the results of the analysis would be vitiated.

Whether permanganate has to be added or not, the contents of the flask are diluted with distilled water to the 100 c.c. mark, and then filtered, *through a dry filter*, into a dry vessel. 50 c.c. of the filtrate are now measured into a beaker, and the amount of silver which they contain determined by means of the decinormal sulphocyanate solution. The number of cc. used is multiplied by two, and the product subtracted from the volume of silver solution employed (*i.e.* 20 c.c.) gives us the amount of silver required to combine with the total chlorine, and therefore the amount of the latter in 10 c.c. of the gastric contents.

b. Determination of the chlorine in mineral combination.

10 c.c. of the mixed gastric contents are evaporated to dryness in a platinum capsule on the water bath. In the absence of a water bath, the capsule may be placed on an asbestos slab, which is heated by means of a gas or spirit-lamp flame, a substitute which permits of the liquid being dried without spurting and therefore without loss. When the residue is dry, it is ignited over the naked lamp, *until the carbonised residue no longer burns with a luminous flame,* care being taken not to ignite the capsule strongly, as chlorides are volatile at a strong red heat.

After the incineration, the residue is moistened and pounded by means of a glass rod; it is treated successively with hot water (in all about 100 c.c.), and the solution is filtered. Care must, necessarily, be taken to ascertain that the whole of the chlorides have been extracted from the carbon. The whole filtrate is then precipitated in a beaker with 10 c.c. of decinormal silver solution, and the excess of silver determined by means of the decinormal sulphocyanate solution. By subtracting the volume of the latter required, from the volume of the silver solution taken (*i.e.* 10 c.c.) we find the amount of pure silver required to combine with the chlorine in the chlorides of the incinerated gastric contents.

Calculation of the hydrochloric acid (a—b).

From the two values (total chlorine *a* and chlorine of chlorides *b*) we ascertain the amount of the total acid present in 10 c.c. of the stomach contents by a simple subtraction. If we multiply the number thus found (the difference) by 0·0365, we obtain the absolute amount of hydrochloric acid in 100 c.c. of the contents of the stomach.

VI.

ON METHYL-MERCAPTAN AS A PRODUCT OF THE PUTREFACTION OF ALBUMINOUS SUBSTANCES AND AS A GASEOUS CONSTITUENT OF THE LARGE INTESTINE.

(*Supplementary to pages* 420, 428, 466.)

Methyl-mercaptan, $CH_3.HS$, is at ordinary temperatures a gaseous body. Under a pressure of 752 mm. at temperatures below 5°.8 C. it condenses. In its liquid form it is a colourless, mobile, highly refracting liquid emitting a fœtid, putrefactive smell. This body, which was first prepared by Gregory[1], and has been carefully investigated by Obermayer[2], and especially by Clason[3], was found by M. Nencki and N. Sieber[4] to be a constant product of the putrefaction of the albuminous substances, and by L. Nencki[5] to be a constituent of the human intestinal gases. Sieber and Schonbenko[6] have since found that when the albuminous substances are fused with caustic potash, methyl-mercaptan is produced in greater quantities than sulphuretted hydrogen. Lastly Rekowski[7] has just examined the physiological action of methyl-mercaptan; he has found it to exert a highly poisonous action on mice, guinea-pigs and rabbits, though the lethal dose appears to be somewhat higher than that of sulphuretted hydrogen.

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[1] Gregory, *Annal. d. Chem. u. Pharm.*, Vol. xv.

[2] Obermayer, *Ber. d. deutsch. chem. Gesellsch.*, 1887, p. 2918.

[3] Peter Clason, *Ber. d. deutsch. chem. Gesellsch.*, 1887, p. 3408.

[4] M. Nencki and N. Sieber, 'Zur Kenntniss der bei der Eiweissgährung Auftretenden Gase.' *Monatshefte f. Chem.*, Vol. x. (1889) p. 526.

[5] Leon Nencki, 'Das Methylmerkaptan als Bestandtheil der menschlichen Darmgase.' *Monatshefte f. Chem.*, Vol. x. p. 862.

[6] Sieber and Schonbenko, *Archives des Sciences Biologiques*, Tome I. p. 315. St Petersburg, 1892.

[7] L. de Rekowski, 'Sur l'action physiologique du Méthylmercaptan.' *Archives des Sciences Biologiques*, Tome II. No. 2, p. 205. St Petersburg, 1893.

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